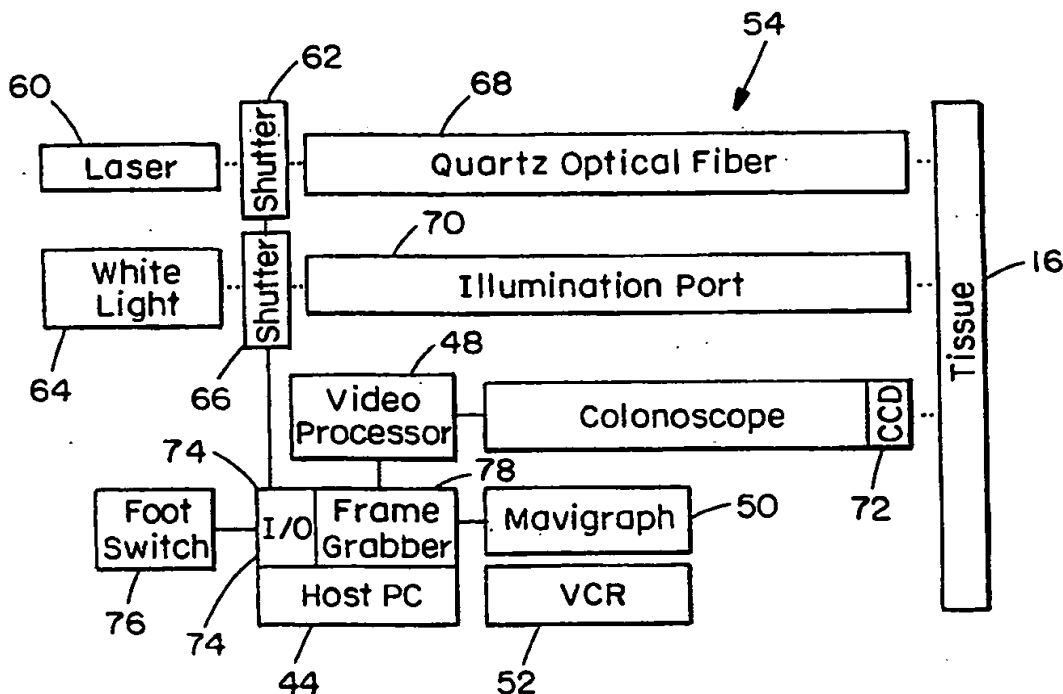




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(54) Title: FLUORESCENCE IMAGING ENDOSCOPE



## (57) Abstract

The present invention relates to a fluorescence endoscope imaging system (40). The system uses first and second light sources (60, 61) to provide fluorescence and reflectance images of tissue (16) being examined. An imaging device (72) mounted at the distal end of the endoscope (54) is used to collect both images.

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## FLUORESCENCE IMAGING ENDOSCOPE

## RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No.60/072,455 filed on January 26, 1998, the entire contents of which is incorporated herein by reference.

5

## Background

The following relates to the development of a laser-induced fluorescence imaging endoscope for mapping cancerous or precancerous tissues in hollow organs. In initial clinical studies, on colon polyps, Ultraviolet (UV) light was used at 370 nm to excite visible fluorescence (400-700 nm), the spectral signatures of which enabled differentiating between normal and abnormal tissues. Previously endoscopic imaging has been achieved using an optics module mounted in one of the biopsy ports of a two-port standard (white light) colonoscope. The optics module employs a quartz optical fiber and associated optics to deliver the UV light to the tissue, and a coherent quartz fiber-optic bundle to transmit the resulting fluorescence image to the proximal side of the endoscope, where a filter removes the large background of reflected UV light and the fluorescence image is then captured by a high-gain CID detector array.

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5           Endoscopically-collected autofluorescence images of  
colonic mucosa can be used as a screening tool for  
detecting pre-cursors to colorectal cancer (CRC).  
Fluorescence has been used to distinguish between normal  
mucosa and adenomas. In particular, spectra measured with  
10          single point contact probes with the use of several  
different excitation wavelengths.

          Fluorescence spectra have been obtained through  
optical fiber probes with several excitation wavelengths.  
An *in vitro* study performed a search over a wide range of  
15          excitation wavelengths, and concluded that 370 nm is  
optimal for distinguishing between normal mucosa and  
adenoma. Both *in vitro* and *in vivo* studies using  
adenomatous polyps as a model for dysplasia have shown that  
with this wavelength dysplasia has less peak intensity at  
20          460 nm and may have increased fluorescence at 680 nm  
compared with normal colonic mucosa. Furthermore, the  
morphologic basis for these spectral differences have been  
studied by fluorescence microscopy. The decreased  
fluorescence intensity in polyps was attributed to its  
25          raised architecture, increased vasculature, and reduced  
collagen in the lamina propria. The red enhancements arise  
from increased fluorescence of the crypt cells, which may  
be caused by higher levels of porphyrin.

### 30          Summary of the Invention

          The present invention relates to imaging endoscopes  
and in particular to a fluorescence imaging colonoscope  
using a dual channel electronic endoscope that employs a

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5 charge coupled device (CCD) chip or other solid state  
imaging device mounted on its distal tip to collect the  
white light image. Of particular significance for the  
present invention is that this chip can also collect the  
fluorescence image, displaying it on the endoscope's video  
10 monitor with much larger signal size than that obtained  
using the optics module and intensified CID camera. This  
configuration was used to collect fluorescence images of  
colonic dysplasia. Video images of two small FAP polyps,  
have been taken with the standard white light image and the  
15 unprocessed fluorescence image.

The CCD detector, which lacks gain intensification,  
detects the weak fluorescence signals, which are six orders  
of magnitude smaller in intensity than the diffusely  
reflected white light image. In addition, it is surprising  
20 that reflected 370 nm excitation light did not completely  
flood the CCD, obscuring the fluorescence signal. This  
results from the fact that the CCD spectral response falls  
off to zero quickly at wavelengths below 400 nm. Thus, the  
CCD effectively serves as its own long pass filter. Other  
25 imaging devices can be used with a filter to reduce by at  
least one half the detected intensity in the ultraviolet  
region relative to the detected intensity in the visible  
region.

In this particular embodiment, the CCD has a  
30 resolution of 270 x 328 pixels and an objective lens of 2.5  
mm in diameter. The images are collected in 33 ms in RGB  
format. The advantages of this particular embodiment  
include that the *in vitro* fluorescence images exhibit a

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5 signal-to-noise ratio (SNR) of about 34 at clinical working  
distances of 20 mm (distance between tip of endoscope and  
tissue surface), which is superior to that obtained using  
the UV Module/CID detector, which has a SNR of about 18 at  
the same distance. The use of the CCD eliminates the need  
10 for the optics module and greatly simplifies system design.  
In addition, it also avoids problems associated with the  
tendency of the UV module to rotate in the biopsy channel.  
By using the same detector and optics for white light and  
fluorescence images, perfect registration of these two  
15 images can be obtained. Parallax between the white light  
image of the CCD and the fluorescence image of the optics  
module was a significant problem. The CCD in this  
particular embodiment contains 88,560 pixels compared to  
10,000 fibers for the UV module, resulting in higher total  
20 image resolution. The objective lens on the Pentax  
colonoscope has better imaging properties than the UV  
module. The characteristic width for the line spread  
function of the lens of this embodiment is 200 mm compared  
to 400 mm for the UV Module. The overall rigidity of the  
25 spectral endoscope is not increased significantly with a  
single UV illumination fiber.

The diagnostic methods employed can be based on the  
overall fluorescence intensity difference between normal  
mucosa and dysplasia. Thus, in certain applications it is  
30 preferable to collect the fluorescence emission over the  
full band between 400-700 nm. However, accurate  
measurements can use a point contact device such that  
diagnostic information can be obtained by sampling the

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5 fluorescence at a plurality of specific wavelengths such as  
460, 600 and 680 nm, for example. For many applications  
the preferred range for fluorescence excitation is between  
350nm and 420 nm. Endoscopic imaging studies with the  
electronic CCD endoscope can include the use of color  
10 CCD's, which have the ability provide such information.

#### Brief Description of the Drawings

Figure 1 is a schematic view of an endoscopic system.

15 Figure 2 is a schematic view of a solid state imaging  
device such as a CCD on the distal end of an endoscope.

Figure 3 is a schematic diagram of an endoscopic  
system in accordance with the invention.

Figure 4 shows the relative sizes of the illumination  
area and fluorescence area.

20 Figure 5 is a schematic diagram of an endoscopic  
system.

Figure 6 is a graphical illustration of the average  
fluorescence intensity and the measured and predicted  
signal to noise (SNR) ratio.

25 Figures 7A and 7B are graphical illustrations of  
variation fluorescence intensity between an average of 14  
frames and a single frame for normal colonic mucosa and  
adenoma, respectively.

30 Figure 8 is an illustration of the sensitivity of the  
system as a function of detection threshold values.

Figures 9 and 10 show fluorescence intensity profiles  
of tissue with adenoma, and including the moving average  
and percent ration values.

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Figure 11 is the fluorescence intensity graph showing adenoma, normal and intensity ratio values as a function of pressure exerted on the site with the probe.

Figure 12 is an endoscope system showing the difference in collection geometry between the endoscope and a contact probe.

Figure 13 is a preferred embodiment of an endoscope system in accordance with the invention.

Figure 14A is a preferred embodiment of a fluorescence imaging system in accordance with the invention.

Figure 14B illustrates graphically the dependence of radiated power on the input power of a light source emitting in the ultraviolet region of the spectrum.

Figure 14C illustrates a timing diagram for a process acquiring fluorescence and reference images.

Figure 15 is a preferred embodiment of a fluorescence imaging system in accordance with the invention.

#### Detailed Description

The equations describing the number of signal photons,  $N_s$ , collected by a given pixel in an endoscope as a function of the separation distance  $d$  and the radial distance  $\rho$  on the tissue surface, and the corresponding SNR are as follows:

$$N_s(p) = \frac{\eta_s \lambda_{em} g^2 T_f T_i T_o f_p r_L^2 \epsilon_i(\lambda_{em}, \Delta\lambda) \tan \theta_m^2 P_o(\lambda_{ex}) \Delta t}{hc \quad 8(1 - \cos \theta_m) N_f d^2 \left( 1 + \left( \frac{\rho}{d} \right)^2 \right)^{3.5}} \quad (1)$$



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$$SNR = \frac{N_s}{\sqrt{N_s + \left(\frac{\sigma_e}{G}\right)^2}} \quad (2)$$

The geometry and certain symbols are defined in Figure 1. Note also, the emission wavelength  $\lambda_{em}$ , pixel array size  $g \times g$ , fiber optic transmission efficiency  $T_i$ , the bandwidth of the filtered emission wavelength  $\Delta\lambda$ , the fraction of the transmitted energy in this wavelength region  $T_f$ , the collective efficiency  $T_o$  of the system optics including the long pass filter, lens and eyepiece, incident light energy  $P_o(\lambda_{em})\Delta t$ ,  $h$  is Planck's constant,  $c$  is the speed of light,  $f_p$  is the packing fraction of the fiber cores  $\epsilon_t$  is the quantum efficiency of the tissue, and  $N_r$  is the total number of resolution elements. The signal to noise ratio (SNR) is a function of electronic noise  $\sigma_e$  and gain  $G$ .

Colorectal cancer constitutes a major national health care problem. The incidence and mortality for carcinomas of the colon and rectum are second only to those of lung in the United States. This suggests that the current screening methods are inadequate for controlling the spread of colon cancer, and that little advancement in detection has occurred in a long time. The five year survival rate for all patients diagnosed is between 35-49%. Colorectal cancer is relatively unresponsive to radiation and chemotherapy, hence surgical resection with wide margins is

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5 the only reliable method of preventing its growth. These tumors spread by direct extension into adjacent structures and by metastasis through the lymphatics and blood vessels. The most common sites of metastatic spread in order are regional lymph nodes, liver, lungs, and bones.

10 The pathophysiology of this disease begins in the epithelial layer of colonic mucosa as dysplastic changes in the crypts cells. This tissue can be accessed by colonoscope, and if the pre-malignant lesions are detected at an early stage, they can be removed for biopsy. Most  
15 carcinomas of the colon and rectum are believed to arise from visible precursor lesions called adenomatous polyps. These benign masses evolve from a monoclonal expansion of epithelial cells which develop irregularities in the size and shape of the nuclei and cytoplasm, a condition known as  
20 dysplasia. These lesions can be detected on colonoscopy by their raised architecture. The medically accepted adenoma-carcinoma sequence suggests that colorectal carcinoma arises from adenomatous tissue that undergoes malignant transformation, which is believed to occur through a multi-  
25 step process in which genetic alterations accumulate. The presence of a precursor stage in the development of CRC provides a window of opportunity for early detection and removal of these lesions to prevent future progression into carcinoma.

30 The prevalent screening method of colonoscopy relies on the observation of large structural changes in the colonic mucosa in order to locate adenomatous tissue for biopsy. However, this procedure is relatively insensitive

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5 to adenomatous tissue which is flat. Patients diagnosed  
with ulcerative colitis (UC), for example, have a high risk  
of developing carcinoma from non-polypoid regions of  
tissue. Moreover recent studies have concluded that some  
10 forms of adenocarcinoma arise from small superficial  
adenomas. Because of this risk, frequent screening by  
colonoscopy must be performed with multiple biopsies  
throughout the colon. However, the likelihood of sampling  
error and missed diagnoses in these patients renders this  
15 form of surveillance highly unsatisfactory. Also, the  
examination of a tissue biopsy is time consuming and  
costly. Moreover, considerable intra- and inter-observer  
variation occurs in the identification of dysplasia. A  
patient who is diagnosed as positive for dysplasia often  
20 must return to the clinic for further screening and  
possibly for surgical resection of the colon. Thus, the  
current state of endoscopic surveillance with histologic  
interpretation is an imperfect science and is in need of  
improved methodologies with greater sensitivity and  
specificity and less intra- and inter-observer variation.

25 The method of fluorescence endoscopic imaging offers  
features which can overcome the present screening  
limitations with white light endoscopy. This method is  
sensitive to the biochemical constituents and  
microarchitecture below the tissue surface. Furthermore,  
30 combined with endoscopes, fluorescence images can scan wide  
areas, and can resolve tissue surfaces on the sub-  
millimeter scale. If sufficient information is present on  
the fluorescence, computers can be used to determine the

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5 presence and location of diseased regions in real-time. Autofluorescence has demonstrated the ability to distinguish between normal and neoplastic human tissue. The first studies showed that single point fluorescence spectra can be used to detect tumors *in vitro* from several  
10 types of tissue,. Later, *in vivo* studies were performed for detecting neoplasia in bladder, brain, colon, cervix, esophagus, lung, oral mucosa, and skin. In addition, fluorescence has been used to distinguish normal tissue from diseased with the use of exogeneous agent such as  
15 hematoporphyrin derivative (HpD).

The full length from the rectum to the cecum is typically 1.5 m. Histologically, the mucosa is the layer in contact with the lumen, and has a thickness of about 400  $\mu\text{m}$ . The epithelium is the most superficial layer and  
20 consists of absorptive columnar cells and intermittent mucin-producing goblet cells, which function to reabsorb water and to lubricate. These cells undergo continuous turnover, and are replaced by rapidly dividing stem cells at the base of the crypts, where the first signs of  
25 dysplasia can be observed. The surrounding lamina propria contains blood and lymphatic capillaries which supports the secretory, absorptive and other highly active functions of the mucosa. It consists of loose connective tissue, in particular collagen, along with numerous inflammatory cells  
30 which protect the intestinal wall from invasion by microbes.

The muscularis mucosa is composed of several layers of smooth muscle fibers which contract to expel secretions

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5 from the glandular crypts, prevents clogging, and enhances  
absorption by maintaining contact between epithelium and  
luminal contents. The submucosa contains the larger blood  
vessels, lymphatics, and nerves, and are surrounded by  
dense collagenous connective tissue which keeps the mucosa  
10 attached to the muscularis propria. The muscularis propria  
contains an inner circular and outer longitudinal muscle  
layer, which are involved in the involuntary peristaltic  
contractions of the colon for propagating the flow of fecal  
matter. The outer serosal layer consists of connective  
15 tissue which contain the major blood vessels and nerves.

Adenomatous polyps are raised protrusions of mucosa  
which contain immature, poorly differentiated epithelial  
cells with irregularity in size and shape of the nuclei.  
These lesions are benign but they have the potential to  
20 transform into colorectal carcinoma. The different  
morphological types include tubular, villous, and  
tubulovillous adenomas. Although all forms are raised,  
each type can either contain a stalk, which is called  
pedunculated, or can be hemispheric, which is known as  
25 sessile. The malignant potential of polyps are greatest  
with the villous form and least with the tubular. Also,  
the probability of carcinoma developing increases with the  
size of the polyp. There is about a 1% chance of finding  
invasive tumor in a polyp less than 1 cm in diameter, 10%  
30 for polyps between 1 and 2 cm, and 45% for polyps larger  
than 2 cm. The sub-cellular changes associated with these  
polyps are frequently histologically identical to the  
dysplasia found in ulcerative colitis.

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5           Results of molecular biology studies suggest that the  
steps involved in the malignant transformation of adenoma  
into carcinoma involves the mutational activation of an  
oncogene coupled with the sequential loss of several tumor  
suppressor genes. Also, it was found that several genes  
10       must incur mutations before malignant tumors arise.  
Several specific genetic alterations have been identified  
during the process of tumorigenesis. Activational  
mutations have been found in the ras oncogene of 50% of  
colorectal carcinoma. Furthermore, allelic deletions were  
15       identified in portions of chromosomes 5, 17, and 18, which  
may involve loss of tumor-suppressor genes.

          Patients with the presence of over 100 neoplastic  
polyps in their colon are diagnosed with the condition  
called familial adenomatous polyposis. These people have a  
20       genetic predisposition for developing numerous polyps in  
their colon by adulthood. Most patients have between 500  
and 2500 polyps, and on average, there are about 100  
polyps. FAP is a rare disease, and accounts for only about  
1% of the incidence of CRC in the Western world. Foci of  
25       dysplasia usually become malignant, and FAP patients must  
have their colons removed at a young age. The probability  
for the onset of colon cancer for someone with this  
condition is 10% at 10 years of age, 50% at 20 years, and  
100% at age 30. Histologically, most of the polyps are  
30       tubular adenomas with a high probability of malignant  
transformation, and the dysplasia associated with FAP  
polyps is identical to that found in sporadic polyps. An  
autosomal dominant genetic defect is responsible for the

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5 development of this disease.

A second form of CRC that is associated with familial predisposition is hereditary nonpolyposis colorectal cancer (HNPCC). HNPCC is defined as patients with at least three relatives in two generations having CRC, and with at least one relative being diagnosed at less than 50 years old. This form is much more common than FAP, and accounts for up to 13% of the incidences of CRC in the Western world. HNPCC patients do not have numerous adenomatous polyps, and it is very difficult to distinguish it from sporadic cases. Genetic linkage has been found between this disease and anonymous microsatellite markers on chromosome 2.

In ulcerative colitis, the mucosa undergoes cytological changes resulting in the formation of dysplasia without the presence of polyp formation. These changes are believed to be associated with repeated episodes of chronic inflammation and repair of the colonic epithelium, and flat, ulcerated tumors with poorly defined margins are common. Patients who have had UC for over 8 years are recommended to have periodic colonoscopy with random biopsies taken. This screening process is not effective because less than 0.1 percent of the total mucosal surface area is sampled. However, it is important to note that only 1% of new incidences of CRC arise from UC cases.

UC is an inflammatory disorder of the colorectal mucosa of unknown cause. Patients with UC are at increased risk for developing dysplasia or cancer. Recognition of this increased risk has resulted in colonoscopic surveillance strategies starting at 7-10 years after the

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5 initial presentation of symptoms. Colonic surveillance  
strategies include direct macroscopic visualization of  
colonic mucosa and access to mucosal biopsies for  
microscopic assessment of dysplasia. Although the  
pathological classification of dysplasia was standardized  
10 in 1983, differences and inconsistencies remain regarding  
the interpretation of dysplasia.

Dysplasia is typically focal. Despite the practice of  
taking 12-20 mucosal biopsies during surveillance  
colonoscopy, less than 1% of the colonic surface is  
15 sampled, so the likelihood of missing small foci of  
dysplasia is high. Thus, cancers can develop in patients  
without any previous or concurrent dysplasia. Although  
performing prophylactic colectomy on all patients after the  
first decade of disease would be the most definitive  
20 solution to the cancer problem in UC, patients with minimal  
or mild symptoms of the disease are understandably  
reluctant to take this radical approach. Colonoscopic  
surveillance with histologic interpretation remains an  
imperfect science in need of improved methodologies with  
25 greater sensitivity and specificity.

Furthermore, studies have suggested that flat  
dysplasia may be the origin of sporadic colon cancer which  
does not arise via the adenoma-carcinoma sequence. The  
morphological characteristics of adenomas that proliferate  
30 superficially in flat nonpolypoid mucosa have been observed  
endoscopically as small plaquelike lesions with vague  
redness or discoloration. In a comprehensive study, 33  
such lesions were described as slightly elevated with a



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5 reddish surface and a central depression. Foci of cancer or severe atypia were found in 25% of lesions of diameter up to 4 mm, 40% of lesions measuring between 5 and 8 mm, and 80% of lesions with diameter between 9 and 10 mm.

10 There are several methods in practice for the early screening for CRC, but each is limited in its effectiveness. The goal of screening is to detect localized superficial masses in asymptomatic individuals.

15 A sigmoidoscopy involves the clinician viewing the patient's rectum and sigmoid colon with either a rigid or flexible imaging device. This form of screening is based on the finding that 60% of CRC occur within the distal 25 cm of the colon. This length is reachable with a rigid sigmoidoscope, and a flexible one can reach up to 60 cm. However, recent statistics have shown that an increasing  
20 number of tumors are found beyond the reach of this device. An advantage of this procedure is that it can be performed without the patient undergoing anesthesia or taking a prep. The most extensive method of screening for this disease is a colonoscopy, where the patient is first prepped and  
25 sedated. A colonoscope is inserted throughout the full length of the colon, and the mucosal surface is viewed by the physician under white light for polyps and other abnormal masses. This procedure is adequate for identifying raised lesions, but flat region of dysplasia  
30 will go undetected.

The fluorescence of tissue occurs through a process in which the electrons of a biological molecule enters an elevated energy state upon absorbing laser light at a given

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5        excitation wavelength  $\lambda_{ex}$ . The excited state is unstable,  
and the electrons will return to the ground state. Most of  
this energy is lost as heat through molecular collisions,  
but a small fraction of excited electrons undergo an  
internal conversion and spontaneously radiates light at  
10       longer emission wavelengths  $\lambda_{em}$ . The fraction of molecules  
which release energy by fluorescence is called the quantum  
efficiency of the tissue, denoted as  $\epsilon$ . The fluorescence  
intensity depends on the product of the initial population  
of the excited state and the tissue quantum efficiency.

15       The spectral lineshape is determined by the  
fluorescence emission and absorption by biochemical  
molecules which are unique to the composition of tissue.  
The electronic levels of the singlet state are split into  
vibrational and rotational states, which in large molecules  
20       consists of small intervals and may overlap due to  
molecular interactions. The electrons may decay to any of  
the vibrational-rotational levels of the ground state,  
thus, the fluorescence spectra of biomolecules are  
typically broad. This lack of structure in the spectra  
25       limits the amount of information that can be obtained from  
fluorescence. The tissue components which produce  
fluorescence are known as fluorophores, and endogenous  
chromophores include aromatic amino acids, NADH, FAD, and  
porphyrins. The local environment may have a large effect  
30       on the fluorescence emission, which may become quenched or  
shifted in wavelength. Further details regarding the use  
of outafluorescence for imaging tissue can be found in U.S.  
Patent Nos. 4,193,142, 5,421,337, 5,452,723, 5,280,788 and

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5 5,345,941, the entire contents of these patents being incorporated herein by reference.

A first step taken in evaluating the use of fluorescence in colon was to determine the existence of optimal wavelengths to differentiate between normal colon  
10 mucosa and adenomatous polyps *in vitro* with single point measurements on a sub-millimeter scale. For example, the fluorescence emissions of 4 normal colon and 11 adenomatous polyps were recorded with a spectrofluorimeter. The excitation wavelengths used ranged between 250 to 500 nm in  
15 10 nm steps, and the results were tabulated in an array called an excitation-emission matrix (EEM). A ratio was taken of the average EEM from the normal colon to that of the adenomatous polyps, and excitation at 330, 370, and 430 nm were found to produce fluorescence spectra which  
20 contained the greatest amount of diagnostic information.

Based on the results of these *in vitro* studies, clinical trials were conducted to evaluate the ability of fluorescence to distinguish among normal, adenomatous, and hyperplastic colon tissue with 370 nm. In this study, a  
25 pulsed nitrogen-pumped dye laser delivered 370 nm excitation through an optical fiber probe with one excitation and six collection fibers. This probe was inserted through the biopsy channel of a colonoscope, and placed in contact with the colonic mucosa during  
30 colonoscopy. The probe consisted of six individual 200  $\mu$ m collection fibers arranged in a bundle with one fiber for excitation. With this device, fluorescence emission was detected from an area of tissue about 1 mm<sup>2</sup>. The

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5 fluorescence spectra were detected by a spectrograph  
coupled to an OMA. The spectra showed a difference at 460  
nm where the normal mucosa produced about 6 times greater  
fluorescence intensity than adenoma. This difference is  
almost twice that found from the *in vitro* studies. Above  
10 650 nm, the average of the adenomas were slightly greater  
than that of normal.

From 20 patients, the fluorescence intensities at 460  
and 680 nm were located on a scatter plot, and a straight  
line was drawn to minimize the number of misclassifications  
15 when compared to histology. The decision line correctly  
classified 31 of 31 adenomas, 3 of 4 hyperplastic polyps,  
and 31 of 32 normal colonic tissue specimens. The  
sensitivity, specificity and positive predictive value of  
the technique for diagnosing adenomas were 100%, 97%, and  
20 94% respectively. Because only a small number of  
hyperplastic polyps were examined, it was unclear whether  
adenoma could be reliably distinguished from hyperplasia  
using fluorescence. The observed differences in the  
fluorescence may arise from architectural differences  
25 between polyps and the normal mucosa rather than from  
dysplastic changes.

The next step was to use the data from this study to  
provide prospective methods of evaluating the performance  
of fluorescence. The data were randomly divided into two  
30 equal sets, and the first was used to devise an algorithm  
to distinguish the tissue type based on the fluorescence  
intensity at 460 nm and at the ratio between intensities at  
680 to that at 600 nm. A biopsy of tissue from each point

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5 was classified histologically as adenomatous, hyperplastic, or normal. From the prospective decision criteria, the sensitivity, specificity and positive predictive value of the algorithm for diagnosing adenomas were 90%, 95%, and 90% respectively.

10 Further attempts have been made to use fluorescence to distinguish between normal mucosa, adenomatous polyps and hyperplastic polyps in vivo with 337 nm excitation. Fluorescence spectra were measured from 86 normal colonic sites, 35 hyperplastic polyps, and 49 adenomatous polyps with a single optical fiber. The fluorescence emission  
15 displayed peaks at 390 and 460 nm, which was attributed to the collagen in the submucosa. Also, this peak decreased in intensity for normal mucosa, hyperplastic polyps, and adenomas, respectively. The peak intensity of the normal  
20 mucosa was found to be slightly less than twice that for adenomas. Using a MVLRL analysis, the sensitivity, specificity, positive predictive value, and negative predictive value of fluorescence to distinguish between adenomatous and hyperplastic polyps were 86%, 77%, 86% and  
25 77%, respectively. This study concluded that the differences in fluorescence were due to polyp morphology rather than to the fluorophores present in the polyps.

Other excitation wavelengths have been used to study fluorescence in colon. A continuous wave He-Cd laser was  
30 used to deliver 325 nm excitation to measure fluorescence spectra from 35 normal mucosa and 35 adenomatous polyps in vitro from a single optical fiber by an OMA. The peak intensity from normal mucosa occurred at 375 nm and that

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5 for adenoma appeared at 450 nm. A multi-variate linear regression (MVLRL) analysis established a set of scores for each data point to determine a diagnostic criterion. Fluorescence spectra from an additional 34 normal, 16 adenomatous, and 16 hyperplastic sites were taken and  
10 analyzed prospectively using the established decision criteria. The sensitivity, specificity and positive predictive value of this study to distinguish between normal and adenomatous tissue were found to be 100%, 100%, and 94%, respectively. In addition, 15 of 16 hyperplastic  
15 polyps were classified as normal, which is the correct diagnosis because hyperplastic polyps are formed from a thickening of the epithelial layer.

The fluorescence of colon was studied with 410 nm excitation as well. The emission from 450-800 nm was  
20 collected with a spectrofluorimeter from 83 biopsy specimens removed during colonoscopy from 45 patients. The intensity of the emission band from 460-530 nm declined from normal to carcinoma to adenomatous mucosa. The peak intensity at 460 nm was about 2.5 times higher for normal  
25 mucosa than for adenoma. A stepwise discriminant analysis was performed on the spectra using nine variables. The results compared to histology showed that the process distinguished between normal mucosa and adenoma with a sensitivity and specificity of 88.2% and 95.2%,  
30 respectively. The fluorescence emission resulted from the superposition of three bands centered at about 470, 485, and 404 nm.

Thus, there has been extensive research performed

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5        within the last 5 to 10 years to evaluate the use of tissue  
autofluorescence to distinguish between adenomatous and  
normal mucosa. In vitro studies have concluded that 330,  
370, and 430 nm are optimal for excitation. Preliminary in  
10        vivo results indicate that single point fluorescence  
detection has a sensitivity, specificity, and positive  
predictive value as high as 90%, 95%, and 90% respectively  
for such discrimination. Also, the data suggest that the  
intrinsic fluorophores can include collagen, NADH, and  
porphyrin. Hemoglobin is an absorbing chromophore. In  
15        order to make this technique suitable for a clinical  
setting, wide area fluorescence detection and processing  
must be performed in real time and adapted to conventional  
white light endoscopy. These requirements demand the  
development of a spectral imaging instrument.

20        Fluorescence microphotographs of unstained frozen  
sections were studied to account for the morphological  
structures in normal colonic mucosa and adenomatous polyps  
which emit fluorescence. The 351 and 364 nm lines from an  
argon-ion laser were used for fluorescence excitation, and  
25        the emission was collected by a series of barrier filters  
with cut-off wavelengths of 420, 475, 515, 570, and 610 nm.  
The fluorescence intensity was graded semi-quantitatively  
from 1+ to 4+ by a single observer. In normal mucosa,  
fluorescence in the spectral band from 420 to 700 from  
30        collagen in the lamina propria was graded at 3+ and that in  
the submucosa at 4+ in the same emission bandwidth. In the  
epithelium, there was faint fluorescence seen from  
absorptive cells and none from goblet cells. The H&E

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5       stained section identifies the tissue composition of normal  
mucosa. The fluorescence image of the serial unstained  
section indicated the fluorescent structures. Several  
differences were observed on the fluorescence image of the  
serial unstained section indicating the fluorescent  
10       structures.

      Several differences were observed on the fluorescence  
micrographs of the adenomatous mucosa. First, fewer  
collagen fibers were present in the lamina propria,  
resulting in less fluorescence intensity from the  
15       epithelium. Also, the level of fluorescence seen in the  
cytoplasm of crypt cells was recorded at 2+, compared to  
+/- seen in normal crypts. Finally, a larger number of  
fluorescent granules were present in adenoma. The image of  
the H&E stained section include crypt cells from an  
20       adenomatous polyp. The fluorescence from the serial  
unstained section shows an observable level of  
fluorescence, and the number of eosinophils in the lamina  
propria is significantly larger than that in normal mucosa.  
The submucosa of the adenomatous polyp was graded at 4+,  
25       which is the same as that of normal.

      A procedure has been developed to describe the  
clinically observed fluorescence in terms of its  
microscopic origins. This process combined the intrinsic  
fluorescence of each microstructure with its density as a  
30       function of tissue depth and the optical turbidity of the  
incident and return path. The concentrations of each  
fluorophore from clinical fluorescence spectra can then be  
extracted. From this procedure, the factors for observing



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5 greater fluorescence intensity from normal mucosa compared  
to that from adenomas include: (1) The submucosal  
fluorescence is about 10 times brighter than that of the  
overlying mucosa. (2) The mucosa attenuates both the  
incoming excitation light and the returning fluorescence;  
10 if the mucosa is sufficiently thick, the underlying  
submucosa cannot contribute, but if it is thin, as in  
normal mucosa, attenuation is smaller, resulting in  
brighter tissue fluorescence. (3) In addition, the  
fluorescence intensity of adenomas is less than that of  
15 normal colonic mucosa, perhaps because the dysplastic  
crypts tend to displace the collagen in the lamina propria,  
which is the dominant fluorophore. (4) Adenomas exhibit  
greater attenuation of both the 370 nm excitation light and  
the return fluorescence, due to increased hemoglobin-rich  
20 microvasculature.

A multi-spectral imaging system has been developed  
which collects fluorescence at four different emission  
wavelengths simultaneously. In this device, the output of  
a fiber optic endoscope is passed through 4 spatially  
25 separated interference filters. The 4 images are arranged  
onto quadrants of an intensified CCD array by adjustable  
segments of a multi-mirror system. The CCD or other  
imaging device 40 as seen in Figure 2 can have 30,000 pixel  
elements or more. The four wavelengths were selected to  
30 optimize the contrast in the fluorescence spectra between  
normal and diseased tissue. Fluorescence from human  
cadaveric aorta was excited with 337 nm, and emissions from  
400, 420, 450, and 480 nm were ratioed to produce a

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5 dimensionless contrast function. This function indicated a  
value for atherosclerotic plaque that was four times  
greater than that for normal artery, and the results were  
displayed using a false-color overlay. This instrument was  
also able to distinguish between rat tumor and surrounding  
10 muscle from fluorescence spectra. Further details  
regarding this system are described in Wang, T.D. et. al.,  
"Real-time In Vivo Endoscopic Imaging of Fluorescence from  
Human Colonic Adenomas", Proceedings of SPIE 1998, 3259,  
the entire contents of which is incorporated herein by  
15 reference.

The resolution of this design is limited by the fibers  
in the imaging bundle. The use of 4 fluorescence emission  
wavelengths provides for greater contrast between normal  
and diseased tissue and for flexibility in the development  
20 of the disease detection process. However, by separating  
the fluorescence emission in parallel, the signal is  
reduced by a factor of 4, thus lowering the SNR. Also, the  
4 spectral images must be aligned onto the detector at  
different angles, which poses a challenge for image  
25 registration. Furthermore, image processing algorithms  
using multiple images increase the computation time, and it  
is not clear that the fluorescence contains independent  
information at 4 bands. Finally, the fluorescence images  
are detected at the proximal end of the endoscope, which  
30 poses difficulty in clinical use for registering the white  
light image and in navigating the instrument.

A simpler version of the multi-spectral imaging system  
has been developed which collects only 2 emission bands.

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5 This design splits the fluorescence emission with a beam  
splitter onto two intensified CCD cameras. A helium  
cadmium laser delivers excitation light at 442 nm via the  
illumination bundle of a fiberoptic bronchoscope. The  
fluorescence emission was filtered in 2 bands, one between  
10 480 and 520 nm and the other at wavelengths greater than  
630 nm. The two spectral images were aligned, and the  
intensities were ratioed point by point for discriminating  
normal from diseased tissue, and a color image was formed.  
This method eliminates the effects of distance and angle of  
15 the illuminating light, as well as tissue reflective  
properties. A color camera is attached separately for  
observing the white light image. This system was tested  
clinically on 53 patients and 41 volunteers, and the  
results were compared with conventional white light  
20 bronchoscopy at 328 sites. The sensitivity on fluorescence  
was 73%, which was significantly greater than that of 48%  
found on white light in detecting dysplasia and carcinoma  
in situ. The two methods were found to have the same  
specificity of 94%.

25 In the clinical system, the white light and  
fluorescence images were collected with a dual-channel  
electronic colonoscope (Pentax EC-3800TL). This model  
contains two biopsy channels with diameters of 3.8 and 2.8  
mm, respectively. The outer diameter of the endoscope is  
30 12.8 mm, and the working length is 1.7 m. The field of  
view of the multi-element objective lens has a divergence  
half-angle of 60° with a depth of focus ranging between 5  
and 100 mm. The white light illumination is produced by a

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5 300 W short-arc xenon lamp. By using the same detector for both white light and fluorescence imaging, perfect registration can be obtained. This feature is ideal for producing a diagnostic overlay.

10 An illumination probe consisted of a 200  $\mu\text{m}$  core diameter optical fiber with NA = 0.40 coupled to a 3 mm diameter BK7 glass microlens ( $F\# = -1$ ). The illumination probe was inserted into one of the instrument channels, and the tip was placed flush with the distal end of the colonoscope. A mode mixer clamped the excitation fiber at  
15 the proximal end to maximize the divergence angle of the light. The probe was attached at the proximal end of the colonoscope by a leur lock to prevent movement. A power of 300 mW was delivered to the tissue. The spectral response of the CCD detector (TI TC210) cuts off at about 400 nm,  
20 and is negligible at the excitation wavelengths  $\lambda_{\text{ex}} = 351$  and 364 nm, thus eliminating the need for a long pass filter to block specular reflection from the excitation light. The two instrument channels allow for the optical fiber illumination probe and the biopsy forceps to be used  
25 at the same time. Figure 1 shows a schematic view of the endoscope 10 with an imaging bundle 20, biopsy view of the endoscope 10 with an imaging bundle 20, biopsy channel 12, lens 18, and illumination ports 14. The distal end of the device is positioned at a distance  $d$  from the tissue. One  
30 problem associated with such a system is the shadows generated by the illumination system. An important feature of the invention described below is a process to compensate for shadows on the tissue 16 surface.

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5           A footswitch was activated by the user to block the  
excitation light when the white light was used for  
illumination, and vice versa, using a pair of computer  
controlled shutters (Uniblitz, VS14). The integration time  
for acquiring each fluorescence image is 33 ms. As shown  
10 in Figure 3, the clinical fluorescence imaging system 40  
consists a video processor 48, computer 44, monitor 46,  
mavigraph 50, and VCR 52, laser 42 and colonoscope 54.

          An electronic colonoscope 54 detects photons at the  
distal end with a CCD detector. An important aspect of the  
15 present invention is that the spectral response of the  
Texas Instrument TC-210 CCD detector dropped sufficiently  
fast below 400 nm that no diffuse reflection from the UV  
excitation was observed. In fact, virtually no specular  
reflection, which is several orders of magnitude higher in  
20 intensity than diffuse reflectance and fluorescence, was  
observed either. Another aspect which made this system  
possible was that the detector has sufficient sensitivity  
to detect fluorescence from colonic mucosa without the use  
of an intensifier. Because the detector is located at the  
25 distal end, the optical transmission efficiency is  
determined only by the multi-element objective lens  
positioned between the detector and the tissue. Another  
significant feature of this embodiment of the invention is  
that the same chip detects both the white light and  
30 fluorescence image, thus perfect registration occurs on the  
pseudo-color overlay. Furthermore, no modifications are  
necessary to the colonoscope which can impede the  
clinician's ability to perform the procedure.

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5           One limitation of this system is the bandwidth  
selectivity and spectral resolution of the chip. The TC  
210 is a monochrome detector and collects fluorescence over  
the full visible spectrum. It is difficult to employ  
bandpass filters in front of the CCD because the light is  
10 collected at angles as high as 60°. However, RGB detectors  
exist which contain pixels which are sensitive to red,  
green, and blue light, and can produce fluorescence images  
in 3 frames. However the passbands are determined by the  
integrated circuit manufacturer of the imaging circuit.  
15 Note that a gating mechanism can also be used, which is  
desirable for using pulsed lasers as the excitation source.  
Other excitation sources can include CW lasers and broad or  
narrow band light sources.

          The block diagram of an electronic imaging system  
20 operated by switch 76 is shown in Figure 5. An argon-ion  
laser 60 delivers UV light through a shutter 62 into a  
quartz optical fiber coupled to a microlens located in one  
instrument channel of the colonoscope, while the white  
light 64 is delivered through shutter 66 the illumination  
25 fibers of port 70. The pair of shutters 62, 55 are  
computer-controlled by a digital input/output (I/O) card  
74. Both the fluorescence and white light images are  
detected by the CCD 72 at the distal end. A frame grabber  
78 digitizes the fluorescence and white light images  
30 sequentially. A host microcomputer executes the image  
processing algorithm and displays the pseudo-color overlay.  
A mavigraph is used to convert the white light image with  
overlay into a format which can be recorded by the VCR.

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5           The plot in Figure 6 shows the fluorescence intensity  
from the average of 14 frames collected with the electronic  
imaging system. A row of pixels is shown from normal  
colonic mucosa. Also plotted are the measured and the  
predicted SNR. The SNR is approximately 30 at the center  
10          and it falls to about 10 near the periphery. Thus, the  
full field of view satisfies the minimum SNR requirement of  
4 for the instrument-noise limited detection for  
distinguishing between normal colonic mucosa and adenomas.

15          A frame-to-frame variation from average in the  
fluorescence image intensities can be seen in Figures 7A  
and 7B, which show the differences between the values  
across a row of pixels in a single frame compared to the  
average of 14 frames. The plot in Figure 7A is that for  
the normal specimen shown in Figure 6, and the plot in  
20          Figure 7B is from a sample of mucosa which contains an  
adenoma in the center. The variation about the average is  
small compared to the difference in fluorescence intensity  
between normal and adenomatous tissue. Thus, the  
occurrence of false positives resulting from pixel-to-pixel  
25          variation is small.

30          A streaking artifact appeared in the fluorescence  
images taken with the electronic imaging system. This  
artifact arose because the UV excitation light was not  
blocked while the CCD rows were being read out  
electronically, which is performed under normal white light  
illumination by a rotating wheel with spatially separated  
filter. This artifact can be removed in the processing  
software of the image data.

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5           A study was performed to determine the level of UV  
light which can be safely delivered onto the colonic  
mucosa. White light and fluorescence images were collected  
sequentially. Fluorescence images from 30 patients with 14  
colonic adenomas and 6 hyperplastic polyps were collected.  
10          Finally, the fluorescence images were collected in parallel  
with single point EEM spectra. From these studies, the  
effectiveness of the realtime implementation of  
fluorescence image collection, processing, and display with  
movement in the colon were assessed. In addition, sources  
15          of artifact present on the colonic mucosa such as mucous,  
stool, and prep were evaluated. Also, the anatomy of the  
colon makes it desirable to collect images at large  
incident angles, and the effectiveness of the moving  
average algorithm with these limitations were determined.  
20          Finally, the intensities from fluorescence images were  
compared to that from the single point optical fiber  
probes.

          The excitation source used was a Coherent Innova 328.  
This laser is rated for 1 W in the UV, and requires 60 A at  
25          208 V of electrical power and 3 gal/min of water. The  
excitation light is coupled into an optical fiber device  
including lengths of 12.5 and 16.5 m of fiber were required  
to deliver the excitation light to the distal end of the  
colonoscope.

30          First, the excitation fiber must be incorporated in  
the colonoscope. Next, a method is used to rapidly switch  
between white light and laser illumination. Finally, a  
method of quickly and accurately registering the



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5 fluorescence results with the white light images must be implemented.

The colonoscopy procedures included prep of the patient with 3 oz of Fleet phospho soda mixed with 4 oz of water. There was no measurable fluorescence from the prep  
10 mixture using an optical fiber contact probe on colonic mucosa *in vitro* with 370 nm excitation.

Using the electronic endoscope, white light reflectance and fluorescence images were collected sequentially *in vivo* during routine colonoscopy. The white  
15 light image can include a vascular pattern of arteries in red, and an outline of a vein in blue. Patches of specular reflection can be seen on the lower half of the images. The fluorescence of normal mucosa appears uniform with an arterial pattern interspersed as reduced fluorescence  
20 intensity. This effect is attributed to the absorption of fluorescence emission by hemoglobin. The vein does not appear on the fluorescence image, and there is virtually no specular reflection from the excitation light. The illumination field on fluorescence is slightly smaller than  
25 that on white light, as depicted in Figure 4.

An example illustrates the process of image collection, processing and evaluation of adenomatous polyps. A white light endoscopic image taken of a sporadic polyp located in the rectum shows a polyp with visible  
30 architectural features about 5 mm in diameter is located in the lower half of the image near the middle. In the raw fluorescence image the adenoma appears as a region of reduced intensity surrounding a brighter central region.

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5           This image was ratioed with its own moving average  
image, and multiplied by 100 to produce the percent ratio  
image. Thresholds on the processed fluorescence images  
taken at 60%, 75%, and 90% were used to determine the  
10          contour lines which define regions of mucosa with various  
likelihoods of containing dysplasia. The contours were  
then filled in pseudocolor to highlight areas of tissue to  
be targeted for biopsy. The pseudocolors red, green and  
blue designate regions on the white light image which have  
high, medium and low probability, respectively. The polyp  
15          was found to be adenomatous on histology.

          Overlay regions indicating disease included one  
located at the site of the adenoma, and the other two  
corresponded to shadows cast by mucosal folds. The shadows  
appeared as regions of reduced intensity on the  
20          fluorescence image. These effects were minimized by  
directing the endoscope normal to the mucosal surface.  
Moreover, the overlay regions which resulted from shadows  
changed in size and shape as the angle of the endoscope to  
the tissue surface varied, while those generated from the  
25          adenoma remained fixed in size.

          White light and fluorescence images were collected  
from a total of 30 patients undergoing routine colonoscopy,  
which included images from 14 adenomas and 5 hyperplastic  
polyps. A biopsy was taken of each adenoma and one  
30          adjacent normal site. The fluorescence images were  
processed by the moving average algorithm, and the  
sensitivity of detection was determined as a function of  
threshold values ranging from 55% to 90%. The results of

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5 sensitivity are plotted in Figure 8.

Autofluorescence images of colonic mucosa can be collected endoscopically *in vivo* and can be used to identify and localize dysplasia in the form of adenomatous polyps. The SNR of the fluorescence images was typically  
10 above 30. The adenomas were correctly identified by the fluorescence algorithm with high sensitivity. As shown in Figure 8, the sensitivity of *in vivo* detection when the images are collected at normal incidence is comparable to that from the *in vitro* studies. At a threshold of 75%, the  
15 sensitivity for detection of colonic adenomas was 86%, compared to that of 92% for the *in vitro* experiments. In order to determine the specificity, the true negatives and false positives must be identified. However, true negatives-(false positives) correspond to regions of normal  
20 mucosa which were found to be normal (diseased) on fluorescence. These results were not obtained because additional biopsies incur additional risk of perforation. Furthermore, the fluorescence from hyperplastic polyps, which are not dysplastic, did not result in regions of  
25 disease from the moving average algorithm.

In comparison of image size, the *in vivo* images encompassed regions of mucosa as large as 10 x 10 cm<sup>2</sup>, whereas the specimens of colonic mucosa were only 2 x 2 cm<sup>2</sup> in the *in vitro* study. In such large fields of view, the  
30 colon contains many mucosal folds, and these layers of tissue blocked the excitation light from reaching the posteriorly-located normal mucosa, thus creating shadows. These folds were not present in the *in vitro* studies.

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5 Diagnostic errors on the processed fluorescence image  
resulted primarily from these shadows. The fluorescence  
method used is based upon the difference in intensity  
between normal and dysplastic mucosa. However, shadows  
appear as regions of reduced intensity without dysplasia  
10 being present. This artifact can be explained by the  
fluorescence excitation geometry. The fluorescence  
excitation is provided by one fiber located in the biopsy  
port for convenience. The center of this instrument  
channel is 8.3 mm away from the center of the CCD detector.  
15 The white light image, on the other hand, is illuminated by  
two fibers whose centers are located only 3.8 mm from the  
detector. Thus, the shadows on the white light image are  
much less pronounced than those on fluorescence.

The fluorescence technique used a single fluorescence  
20 emission band for detection of adenomas. This method  
worked well in vitro when the colonoscope is placed at  
normal incidence to the lesion, and no mucosal folds were  
present. However, during the clinical use of the  
fluorescence prototype, the view of the endoscope was often  
25 limited to the side of the adenoma. Because the colon is  
a tube-shaped structure, some adenomas were anatomically  
located at sites where it was virtually impossible to  
orient the colonoscope at normal incidence to the lesion.  
As a result, one side of the lesion may not be surrounded  
30 by normal colonic mucosa. Another situation was that the  
normal mucosa is far away to produce fluorescence  
intensities sufficiently higher than that of the adenoma.

The fluorescence intensities were measured from the

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5 raw images. The normalized intensity values and the  
intensity ratios were taken at three sites within the  
adenoma (denoted by left, center, and right in Table 3).  
The plot in Figure 9 contains fluorescence intensity  
profiles through the adenoma, representing the raw  
10 fluorescence and percent ratio values, respectively. The  
adenoma was approximately 8 mm in diameter. On  
fluorescence, the lesion is located between the 11 mm and  
the 19 mm markings on the abscissa, which are labeled by  
the vertical lines near the x-axis in Figure 9. Most of  
15 the adenomas exhibited a single fluorescence intensity  
minimum at the center of the lesion; the average ratio  
between normal and diseased pixels was  $1.8 \pm 0.5$  at the  
center, and  $2.0 \pm 0.6$  and  $2.0 \pm 0.7$  at the left and right  
midpoints, respectively. The average intensity ratio at  
20 these sites was  $2.0 \pm 0.6$ . The results of this procedure  
show that the differences between normal colonic mucosa and  
adenomas for *in vivo* fluorescence images are very similar  
to that *in vitro*.

Similarly, the fluorescence intensities were measured  
25 from the raw images for hyperplastic polyps. The  
normalized intensity values and the intensity ratios were  
taken at three sites within the polyp (denoted by left,  
center, and right in Table 3). The plot in Figure 10 shows  
the fluorescence intensity profiles through the  
30 hyperplastic polyp, representing the raw fluorescence and  
percent ratio values, respectively. The hyperplastic polyp  
was approximately 5 mm in diameter. On fluorescence, the  
lesion is located between the 17 mm and the 22 mm markings

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5 on the abscissa, which are labeled by the vertical lines  
near the x-axis in Figure 10. The hyperplastic polyps  
exhibited an approximately uniform fluorescence intensity  
across the lesion which was continuous with the normal  
colonic mucosa. The average ratio between normal and  
10 diseased pixels was  $1.1 \pm 0.1$  at the center, and  $1.2 \pm 0.1$   
and  $1.1 \pm 0.2$  at the left and right midpoints,  
respectively. The average intensity ratio at these sites  
was  $1.1 \pm 0.2$ . Because this average ratio value is not  
significantly different from that of normal mucosa, it is  
15 not surprising that no region of disease could be  
identified by this intensity method.

In the *in vivo* images, the vascular pattern was  
clearly displayed on both the white light and fluorescence  
images. The vessels were not apparent on the *in vitro*  
20 images, perhaps because the blood supply of the living  
colon was no longer intact. The hemoglobin in the blood is  
a well-known absorber of light, and produces linear  
patterns of weak fluorescence intensity. Thus, the  
intensities were measured from the raw fluorescence images  
25 of blood vessels. As shown in Table 3, the intensity ratio  
from the blood vessels is  $1.3 \pm 0.1$ . This value is  
significantly less than the average from adenomas, thus  
blood vessels will not present as a source of artifact on  
the overlay. Furthermore, image processing methods can be  
30 used to remove the blood vessels based on their shape. In  
Table 3, the intensity ratios for adenomas, hyperplastic  
polyps, and blood vessels are summarized for comparison.

Endoscopic images and single point spectra can both

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5 provide valuable information about tissue biochemistry.  
Each method has its own advantages and disadvantages. The  
endoscope collects images, and provides spatial information  
with sub-millimeter resolution. The fluorescence intensity  
between normal mucosa and adenomas can be compared from the  
10 same image field within a fraction of a mm from each other.  
Also, fluorescence images are collected remotely, thus the  
pressure on the tissue is uniform throughout the image  
field. However, it is more difficult to acquire spectral  
information with fluorescence. Because of the larger areas  
15 involved, the fluorescence energy may become too weak at  
each pixel to maintain sufficient SNR, unless very large  
excitation power is used.

On the other hand, single point optical fiber contact  
probes collect fluorescence from an area of approximately 1  
20 mm in diameter only. With an intensified optical  
multi-channel analyzer (OMA), spectra over a wide bandwidth  
can be measured with good spectral resolution and high SNR.  
However, the probe must be placed at several sites on the  
mucosa to sample differences between normal and adenoma.  
25 Typically, the normal mucosa sampled is several cm away  
from the adenoma, and comparisons of the absolute intensity  
can be affected by biological variability over distance.

The degree of contact of the probe on the polyp can  
vary during the *in vivo* measurements because the colonic  
30 musculature is constantly contracting and expanding. As a  
result, movement is created which makes probe placement  
difficult. The adenoma is round and slippery, and the  
movement of the colonic wall renders complete contact with

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5 the surface of the polyp very difficult. Furthermore, the distal end of the optical fiber probe is not flat, but there is a 17° bevel. Thus, the orientation of the beveled side will affect the degree of contact as well.

10 Results of the colonoscopy procedure showed that it was very difficult to place the probe onto the polyp for the 0.5 seconds required to collect a full EEM. Light escaping at various colors representing the excitation sources was observed on the normal mucosa surrounding the adenoma. This observation suggests that the delivery of  
15 excitation energy to the polyp and collection of fluorescence emission was not complete. Probe contact was hindered by the physiological movement of the mucosa, and by the fact that a flat probe was being placed on a slippery, hemispherical surface. Contact is not a problem  
20 for spectra collected on normal mucosa because this surface is flat.

Moreover, the ratios between the intensities of normal mucosa and adenomas can be affected by difference in the pressure exerted on each site. An in vitro experiment was  
25 conducted on a resected specimen of colonic mucosa which contained an adenoma. The fluorescence intensity in the spectral range between 400 and 700 nm was measured as a function of pressure exerted by the probe which was passed through the biopsy channel of a colonoscope. The pressure  
30 was measured with a balance. As shown in Figure 11, the fluorescence intensity increases with pressure, and the intensity ratio does not change if equal pressure is exerted on both the normal and adenoma sites. However,



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5       this is usually not the case during the clinical  
acquisition of spectra. The normal mucosa is relatively  
flat, and measurements can be made with virtually complete  
probe contact with a few grams of pressure. On the other  
hand, the pressure on the polyp cannot be made the same as  
10       that on the normal site because the probe will slip off.  
The pressure on the normal site was estimated to be about 5  
grams, while that on the adenoma was estimated to be close  
to zero. Thus, the difference in pressure exerted on the  
normal mucosa and the adenoma may result in the intensity  
15       ratio increasing from 2 to 3, as shown in Figure 11.

Furthermore, on the recorded images of the colonoscopy  
procedures, the normal mucosa showed an indentation at the  
site where the probe was placed during the collection of  
spectra. This observation confirmed the estimate that  
20       several grams pressure was exerted on normal mucosa during  
data collection. On the other hand, the probe was seen to  
slide off the polyp when any significant pressure was  
exerted, which resulted from the moistness of the surface.  
Thus, the pressure exerted on adenomas was significantly  
25       less.

Another procedure was conducted *in vitro* to compare  
the fluorescence intensity ratio between normal mucosa and  
adenoma as measured on imaging and single point. White  
light and fluorescence images of a resected specimen of  
30       colonic mucosa containing two adenomas were obtained. The  
intensities were measured from 7 normal sites immediately  
adjacent to the adenomas on both imaging and single point.  
The results included the intensities that were normalized

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5 so that the average value is 100 for each system. This  
step allows for direct comparisons to be made at each  
point, and reveals that the intensities are within about  
10% of each other. Furthermore, the normalized intensity  
values range from 68 to 155 on imaging and from 63 to 136  
10 on single point. Thus, the intensities measured on normal  
mucosa depend on the site sampled with both methods, and  
can vary by over a factor of 2.

In Table 4, the normalized intensities and the  
intensity ratios are determined for the two adenomas on  
15 imaging and single point. These values are determined at  
the center and the left and right midpoints of the  
adenomas. For the left adenoma, the average intensity  
ratio was 1.43 on imaging and 1.54 on single point. For  
the right adenoma, the average intensity ratio was 1.52 on  
20 imaging and 1.72 on single point. These results indicate  
there is little difference in the intensity ratios between  
imaging and single point *in vitro*.

The fluorescence intensity ratio was calculated from  
Monte-Carlo simulations to determine the fluorescence  
25 intensity ratio, given the different excitation and  
collection geometries of the imaging system and single  
point. In Figure 12, a diagram of the collection geometry  
for the endoscope 100 and the single point probe 102 is  
shown. The endoscope contains a 2.5 mm diameter objective  
30 lens 104, and is located in air at a distance 20 from the  
surface of the tissue. This geometry corresponds to a  
collection angle of 40°. The probe contains a quartz  
shield 106 which is in contact with the tissue 16. The

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5 optical fibers are located at a distance of 2 mm from the tissue surface by this shield 106, and collect light at a NA = 0.22, which corresponds to a collection angle of 12.7°. The optical parameters of colonic mucosa for the excitation and emission wavelengths are shown in Table 2.

10 The excitation used in the simulation is an infinitely-thin beam with a divergence angle of 0°. The fluorescence intensity at a point on the tissue from a uniform thick excitation beam can be determined from the fluorescence collected from a superposition of  
15 infinitely-thin excitation beams which are incrementally displaced in distance from the point to be measured. However, this result is equivalent to integrating the fluorescence intensity over the field of view. The LSF of the tissue falls off quickly within several mm; thus the  
20 simulation integrates over a 2 mm region within the collection angle specified in Table 5. The results of the simulation are shown in terms of the intensity ratio between the light collected at the tissue surface with that of the excitation. In Table 5, the intensity ratio between  
25 normal colonic mucosa and adenomas is 3.0 and 2.9 for the endoscope and the probe, respectively. The intensity ratio is similar for the endoscope and the probe, a result which is consistent with the *in vitro* studies. The intensity ratio for the endoscope is slightly higher than that of the  
30 probe, which is consistent with the collection angle of the endoscope being smaller. Light from the highly fluorescent submucosa is more likely to reach the detector with a smaller collection angle.

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5           A model was developed to quantify the number of  
photons collected by the endoscopic imaging system over the  
field of view at normal angle of incidence. This result is  
valid for both white light reflectance and fluorescence  
images, and can be applied to both the fiber optic imaging  
10 bundles and electronic imaging systems. The spatial  
distribution of the illumination and emission profiles of  
in the center and to fall off towards the periphery of the  
image. When combined with the detector noise statistics,  
the SNR of the image can also be determined. This analysis  
15 showed that distance and optical collection geometry  
produces a profile in which the SNR at the periphery was  
always lower than that in the center. This parameter is  
needed for developing algorithms for identifying tissue  
lesions. Also, the collected light intensity was found to  
20 decrease with the square of the distance between the distal  
end of the endoscope and the tissue. Furthermore, the  
light collection by coherent imaging bundles is limited by  
the numerical aperture of the optical fiber. This analytic  
tool can be used to design the optical parameters of the  
25 fluorescence imaging system and to identify the type of  
light source required to excite the fluorescence.

The methods developed for endoscopic imaging model  
were used to determine the excitation source, optics, and  
detectors necessary for building two fluorescence imaging  
30 systems. The first design consisted of a fiber optic  
colonoscope which detected the fluorescence image at the  
proximal end with an intensified CID camera. A 400 nm long  
pass filter was used to block the reflected excitation

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5 light, an a quartz optical fiber located external to the  
colonoscope was used for image excitation. The second  
design was a modification of the first to accommodate the  
requirements for clinical use. This system used an  
electronic colonoscope with dual instrument channels, and  
10 detected fluorescence images at the distal end. The cutoff  
in spectral sensitivity of the CCD detector below 400 nm  
was used to avoid the reflected excitation light. An  
illumination probe with a high NA quartz optical fiber was  
coupled to a microlens and inserted into one instrument  
15 channel for image excitation. In both systems, the  
excitation source was an argon-ion laser which delivered  
about 300 mW at  $\lambda_{ex} = 351$  and 364 nm, and microcomputer  
with a frame grabber was used to acquire, process, and  
display the diagnostic images.

20 Autofluorescence images from human colonic adenomas  
were collected with the fiber optic system with high SNR in  
vitro. For wide area surveillance of the colon wall,  
regions of mucosa as large as 100 mm<sup>2</sup> must be illuminated.  
Furthermore, the endoscopic images are collected remotely,  
25 and the intensity collected falls with distance d squared.  
Previously, fluorescence spectra were collected from  
contact probes which illuminated an area of about 1 mm<sup>2</sup>.  
The results of this study showed that excitation sources,  
optics, and detectors used in this design could collect  
30 autofluorescence images with sufficient SNR to distinguish  
between normal colonic mucosa and adenomas. In the fiber  
optic system, an SNR of over 30 was attained, which  
exceeded the minimum SNR requirement of 7.

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5        Fluorescence images were then collected from samples  
of resected colonic mucosa *in vitro* to evaluate the  
potential use of this technique for wide area surveillance  
of dysplasia Colectomy specimens from three patients with  
familial adenomatous polyposis containing polypoid and  
10       non-polypoid adenomas were studied. Each raw image was  
corrected for differences in distance and instrument light  
collection efficiency by normalizing to a spatially  
averaged image. Intensity thresholding was then used to  
identify diseased regions of mucosa. The sensitivity and  
15       specificity for detecting a region of dysplasia depended on  
the threshold value selected. With the threshold set to  
75% of the average normal intensity, a sensitivity of 90%  
and a specificity of 92% were achieved. The average  
fluorescence intensity from normal mucosa was found to be  
20       greater than that from the adenomas by a factor of  $2.2 \pm$   
 $0.6$ . These results demonstrate the potential of this  
technique to direct biopsy site selection.

      The results from the *in vitro* studies provided  
motivation for conducting an *in vivo* study. The electronic  
25       system was used to collect autofluorescence images from  
colonic adenomas *in vivo*. In the this system, an SNR of  
over 30 was attained as well, which exceeded the minimum  
SNR requirement of 4. Fluorescence images were collected  
from 14 adenomas and 6 hyperplastic polyps from 30 patients  
30       undergoing routine colonoscopy. The fluorescence images  
were collected in a 33 ms frames, and were processed by  
dividing the raw fluorescence image with a moving average  
image. The processed images displayed regions of mucosa

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5 with a probability of containing dysplasia in the form of  
adenomas, as verified on histology. With the threshold set  
to 75% of the average normal intensity, a sensitivity of  
86% was achieved for detecting adenomas and a specificity  
of 100% was attained for hyperplastics. On average, the  
10 ratio between the fluorescence intensity of normal mucosa  
to that from adenomas was  $2.0 \pm 0.6$  and to that from  
hyperplastic polyps was  $1.1 \pm 0.2$ . The diseased regions on  
fluorescence best corresponded to the adenoma on white  
light when the colonoscope was at normal incidence. At  
15 higher angles there were greater effects from shadows.  
These results showed that dysplasia can be identified on  
fluorescence images *in vivo*.

In the single point optical fiber contact probe  
studies the average intensity ratio between the  
20 fluorescence at 460 nm from normal colonic mucosa and  
adenomas was found to be about 3, while that in endoscopic  
imaging this ratio was measured to be  $2.0 \pm 0.6$ . Direct  
comparison of fluorescence imaging and single point  
measurements *in vitro* revealed that there was little  
25 difference between the intensity ratio measured on imaging  
compared to that measured from single point. There are two  
possibilities that can account for the difference in  
intensity ratio between the two methods. First, the ratio  
of 3 measured by the single point method was performed *in*  
30 *vivo*. A lower ratio may have resulted *in vitro* because of  
the loss of blood flow, which is known to absorb light.

Alternatively, the difference in the ratios may result  
from contact and pressure artifacts. Videotapes of the

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5 colonoscopy procedure showed that it was very difficult to  
place the probe onto the polyp for the 0.5 seconds required  
to collect a full EEM. Light at various color representing  
the excitation sources was observed, which indicated that  
the delivery of excitation energy to the polyp and  
10 collection of fluorescence emission was not complete.  
Probe contact was hindered by the physiological movement of  
the mucosa, and by the fact that a flat probe was being  
placed on a slippery, hemispherical surface. Contact is  
not a problem for spectra collected on normal mucosa  
15 because this surface is flat. Furthermore, increased  
pressure was found to elevate the fluorescence intensity  
collected. Higher pressures were exerted on the normal  
mucosa compared to that on the polyp. The probe was seen  
to slide off the polyp when any significant pressure was  
20 exerted. Both differences in contact and pressure *in vivo*  
resulted in a higher ratio between normal mucosa and  
adenoma. On the other hand, the fluorescence images are  
collected remotely, and the pressure and contact parameters  
are identical for normal mucosa and adenoma.

25 Finally, the results of the clinical studies  
identified future directions to improve the sensitivity and  
clinical usefulness of fluorescence endoscopic imaging.  
The shadow artifact can be reduced by illuminating the  
tissue through the two white light ports. This  
30 modification can be accomplished by replacing the glass  
fibers with quartz, thus allowing for both white and  
excitation light to be transmitted. Furthermore, the  
shadow artifact, angle of incidence, and detection yield



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5 can all be improved by collecting multi-spectral images consisting of two or more fluorescence images. Lastly, the concurrent collection of EEM spectra can be used to identify new excitation wavelengths which result in higher intensity contrast ratios.

10 Dysplastic tissue exhibits an increase in red fluorescence which can be detected to improve the sensitivity of disease detection. Thus another embodiment includes the collection of multiple emission wavelengths. One method of collecting multiple fluorescence emission  
15 wavelengths is to use an electronic endoscope (e.g. Olympus, Model CF I OOTL) with a CCD detector which is sensitive to the red, green, and blue (RGB) regions of the visible spectrum. Fluorescence images from each RGB frame can be captured and processed, providing more detailed  
20 information for use in a diagnostic procedure. Furthermore, the use of spectral lineshape information from images at different wavelengths reduces all geometric distortions. The TI TC244 has a quantum efficiency of 30% at 640 nm and 15% at 480 nm [TI Manual, 1994].  
25 Extrapolating from the 370 nm imaging data and the EEM data, a SNR of 10:1 in the red and 50:1 in the blue is anticipated.

Performing the detection on the distal end of the electronic colonoscope has many practical advantages.  
30 First, the same detector can be used for both white light and fluorescence imaging. A single detector not only results in perfect registration of the two images, but avoids the need to interchange of cameras, which can be

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5       cumbersome. Second, fewer optical elements results in a  
transmission efficiency of fluorescence photons which is  
significantly higher than that of a fiber optic imaging  
bundle. Third, the packing geometry of CCD pixels allow  
for minimal loss of surface area of detection, unlike fiber  
10       optic imaging bundles which have a hexagonal packing array.

While there are advantages to detecting the  
fluorescence image with a distally located CCD, a fiber  
optic imaging bundle with proximal detection has advantages  
as well. The spectral bands of the distal CCD is limited  
15       to the RGB response of the distal detector, while the  
fluorescence collected by a fiber optic imaging bundle  
could be filtered into an unlimited number of spectral  
images. Also, detection of the fluorescence image at the  
proximal can allow for detection with a gated intensifier.  
20       This device enables use of pulsed lasers.

The EEM study provides valuable guidance about new  
imaging strategies. The results indicate that excitation  
near 410nm is useful. The contrast between normal and  
adenoma tissues provided by the blue fluorescence is  
25       greatly enhanced compared to that obtained with our current  
excitation wavelength (10:1 versus 2:1). In addition, the  
red fluorescence is quite pronounced for adenoma.  
Extrapolation of the conclusions of the morphological model  
developed using  $\lambda_{ex} = 365 \text{ nm}$  to this new excitation  
30       wavelength suggests that the blue fluorescence contains  
information about both crowding of the crypts and mucosal  
thickness, and that the red fluorescence contains  
information about crypt cell dysplasia. Hence, collecting

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5 images at red and blue emission wavelengths should provide  
both high contrast diagnostic images and significant new  
histological information. In addition, the ratio image can  
be used to normalize out shadow effects. The next phase of  
the imaging studies will use 410 nm excitation. A krypton  
10 ion laser (Coherent Innova Model 302) will provide 500 mW  
of power at the two lines 407 and 413 nm. This level of  
power is adequate to achieve large fluorescence signals in  
both red and blue bands. This laser will be installed at  
the BWH Laser Laboratory along with the existing 365 nm  
15 argon ion laser.

In addition, multiple excitation wavelengths can be  
employed. One approach would be to use excitation from the  
407 and 413 nm lines of a krypton ion laser to excite the  
red fluorescence and to retain the 365 and 351 nm lines  
20 from argon ion laser to excite the blue fluorescence. Two  
hardware configurations include (1) a fiber endoscope with  
a switchable filter wheel between the scope and camera, and  
(2) a dual-chip endoscope. Such a system has been  
developed, for example, by American Hospital, Inc., for  
25 stereo viewing during endoscopy. One can modify one of the  
windows on the chip with a spectral cut-off mechanism. The  
timing of the red-sensitive imaging channel can be  
synchronized with the excitation light.

The diffuse reflectance image at 407-413 nm can be  
30 explored to obtain information about the tissue hemoglobin  
content. This image can be obtained by installing a filter  
with the appropriate bandwidth on the rotating wheel in  
front of the white light source. The approach is to ratio

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5       this reflectance image with the fluorescence images in the red and blue frames. In order to develop the required algorithms, and to decide how to optimize the spectral information collected, an extensive contact probe study with 410 nm excitation can be performed.

10       The shadow artifact obtained using the broadband intensity algorithm with 365 nm excitation can be greatly reduced by use of an improved excitation geometry. Currently, excitation light is delivered through a single quartz fiber located in the biopsy channel located 8.3 mm from the CCD detector. The use of a single illumination beam located a large distance from the CCD chip tends to enhance shadows. In contrast, in the conventional white light images produced by this colonoscope, shadows are minimized by use of two closely spaced white light illumination beams symmetrically positioned on opposite sides of the CCD chip. By replacing the illumination fibers with quartz fibers, the UV light can be delivered through the two white light illumination ports, which are located only 3.8 mm from the CCD detector. Implementing this requires modifying the video processor to enable alternate coupling of white light and laser excitation into the illumination fibers.

25       Other spectral endoscope improvements can include: (I) regulating the excitation light intensity on the tissue surface via feedback control. This provides constant illumination, regardless of viewing distance, and is also important for patient safety; (ii) minimizing the streaking effect of the fluorescence excitation on the white light

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5 endoscopic imaging display by timing the fluorescence  
excitation to occur during the "blank" periods of the  
filter wheel used in the endoscope white-light source.  
Feedback control of the excitation light can be  
accomplished by measurement of the average intensity on the  
10 fluorescence image. The intensity of this average value  
will be used to modulate the open period on the shutter or  
filter wheel. The streaking effect can be completely  
removed by implementing the identical filter wheel for  
blocking the excitation light that is used for producing  
15 the RGB illumination on the white light mode.

As described above, a large argon-ion laser was used  
as a near-LTV excitation source for the imaging studies.  
Although adequate for these studies, this light source is  
expensive and bulky and operates at only a few discrete  
20 wavelengths. Such a laser system with its special  
electrical and water cooling requirements cannot be easily  
moved, preventing use at multiple sites. Alternative  
excitation sources can be considered which include a pulsed  
laser and a white-light source with filters, both of which  
25 are compact and transportable.

For applications in which near-UV excitation is  
appropriate, a pulsed ND:YAG laser is used because it can  
provide third harmonic radiation at 355 nm with sufficient  
average power for spectral imaging. In both the *in vitro*  
30 and *in vivo* studies, good SNR was obtained with 300 mW of  
laser power, which corresponds to 10 mJ of energy per  
pulse. Therefore, a frequency tripled ND:YAG laser with a  
5 - 10 ns pulse duration operating at 30 Hz with an average

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5 power of 300 mW at 355 nm will be adequate. Using a CCD  
camera gated at about 10 ns, this short excitation pulse  
enables simultaneous acquisition of white light and  
fluorescence images. Within this short temporal gate the  
white light background is negligible, obviating the need to  
10 chop the white light illumination. There are no special  
power or water requirements for lasers of this type and a  
fluorescence endoscope system with such a laser will be  
easily transportable.

A mercury lamp can also be used as an excitation  
15 source. Such a source is compact and lightweight and can  
provide a bright, narrowband illumination at a number of  
excitation wavelengths. Employing this light source  
simplifies system design and reduce cost, enabling less  
expensive units to be produced for use at multiple sites.  
20 The key issue is whether enough light in the desired  
wavelength range can be coupled into the illumination  
fiber(s). A commercial white light source with a 150 W  
xenon lamp is capable of delivering as much as 80 mW of  
white light at the distal end. Utilizing a 50 run  
25 excitation bandwidth, about 20 mW of light can be used to  
induce tissue fluorescence.

At selected wavelengths, mercury lamps have 5 to 10  
times higher output powers than that of xenon. This  
indicates that with a 500 W mercury lamp having a  
30 relatively small filament, at least 300 mW of useful  
excitation light should be available at the distal end of  
the illumination fibery should be sufficient for collection  
of good quality fluorescence images from colonic tissue.

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5 In addition, to further enhance SNR, either the total area of illumination can be reduced or imaging elements can be binned together. A lamp and power supply can be selected for this application with the proper brightness, stability and minimum electrical interference.

10 Currently, the image processing scheme is based on ratioing the raw image to a spatially-averaged image, and applying a threshold criterion for classifying a region of tissue as normal or diseased. The averaging window and detection threshold values are pre-flexed, regardless of  
15 the polyp size, viewing angle and distance. These predetermined values limit the range of polyp sizes which can be accurately measured. Improved image processing and thresholding methods will employ variable window sizes for spatial averaging and variable thresholds. Information  
20 from the raw digitized image about the diameter of the largest lesion in the image will be used to determine these parameters. This change in the window size as a function of the lesion in the image field will maximize the intensity ratio and optimize the performance of the  
25 fluorescence method.

Image analysis methods for extracting information from multivariate images can also be explored. A multivariate image is a collection of congruent images of the same object measured with different variables, such as reflected  
30 wavelengths, or fluorescence or Raman band intensities. Many methods are available for analyzing multivariate images, and they can be adapted to image analysis. In general, three steps will be followed, image processing,

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5        object segmentation, and contrast measurement. The images  
will first be processed based on the selected operation,  
such as moving-window average, intensity difference or  
ratio. The processed image will then be segmented based on  
both frequency and intensity information. This can be done  
10       either through thresholding, quick/slow descent, or region  
growth. These methods can be coupled to the concomitant  
identification and display of a lesion(s) based on a  
probabilistic scheme.

      When techniques for collecting multiple spectral  
15       images are developed and a database of such images are  
built, more advanced image analysis methods, such as  
principal component analysis and regression analysis can be  
used. Principal component analysis does not assume a known  
(a priori) distribution, but instead employs a set of  
20       calibrated data to extract information about structures  
exhibiting pre-malignant changes. The regression technique  
is based on the principle of building up a mathematical  
relationship between two groups of variables, i.e., between  
a number of independent variables and one or more dependent  
25       variables. As an example, a logistic regression to  
correlate spectral intensities in the images with  
histopathology of dysplastic lesions.

      The development of the fluorescence imaging endoscope  
has demonstrated the potential to perform wide-area  
30       surveillance colonoscopy using fluorescence. The  
fluorescence image can be analyzed in real time and can  
provide the endoscopist with an instant interpretation of  
the probability of dysplasia determined using a



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5 previously-validated algorithm. In addition, the ability to guide biopsy can be used with the present invention. In patients with FAP, fluorescence imaging can be used to direct mucosal biopsies to areas that are endoscopically normal-appearing (non-polypoid) but, based on their  
10 spectral characteristics, can have an increased likelihood of being dysplastic. Histopathological assessment of mucosal biopsies will be correlated with spectral data to validate for detection of "flat" dysplasia.

The following method can be followed for determining  
15 the capability of the fluorescence imaging system for directing biopsy. The entire surface of the colon wall, both at colonoscopy and using resected samples at colectomy, is systematically imaged, and isolated areas which are diagnosed as dysplastic selected for directed  
20 biopsy. Random areas diagnosed as benign can also be sampled, and the spectral diagnosis confirmed by histological analysis. Again, the effects of complications such as inflammation can be investigated. Once an imaging algorithm has been validated, it can be adapted to the  
25 detection of dysplasia in patients with UC. As in the case of the contact probe studies, diagnostic algorithms for UC must be capable of evaluating patients with various degrees of background inflammation. The same patient groups studied with contact probe EEMs will be studied with  
30 fluorescence imaging. An important potential benefit of wide area fluorescence surveillance is that one or more of the otherwise random biopsies obtained during conventional surveillance colonoscopy may be directed by the results of

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5 fluorescence imaging. Those biopsies can be separated from the remainder of the random biopsies to assess whether fluorescence imaging can increase the yield of dysplasia detection over random sampling.

10 The development of the rapid EEM and spectral imaging systems represent two very important advances in instrumentation. The two systems are complementary. The imaging system views wide areas of mucosa in real time, and the EEM system provides complete spectral characterization of a given site of colonic mucosa. The two instruments can  
15 be used simultaneously, where appropriate. The EEM probe is placed through the second channel of a two channel colonoscope. Thus, each system can be used to verify the other. Also incorporated herein is the publication attached hereto and entitled "Real-Time in vivo endoscope  
20 imaging of fluorescence from human colonid adenomas".

The following table compares the signal size expected for white light imaging, fluorescence imaging observed with the endoscope CCD, and fluorescence imaging using the optics module with the intensified CID camera. The parameters listed below are taken from either the manufacturer's specification or from experimental measurements.

<u>Imaging Device</u>	<u>Definition</u>	<u>Pentax(white light)</u>	<u>Pentax(fluorescence)</u>	<u>UV</u>
<u>Module</u>				
$\lambda_{ex}$ (nm)	ex wavelength	-	356	356
$\lambda_{em}$ (nm)	em wavelength	-	460	460
$\Delta\lambda$ (nm)	em bandwidth	400-700	400-700	400-700
$P_o$ (mW)	power	1	300	300
$\Delta t$ (s)	integration time	0.011	0.011	0.033
$d$ (mm)	distance	20	20	20
Diameter (mm)	area illum	70	70	28
$\theta_m$ (degrees)	max angle	60	60	35
$N_f$ (pixels/fibers)	number	88560	88560	10000
$r_L$ lens (mm)	radius	1.25	1.25	0.3
$\epsilon_t$	tissue efficiency	1	5.00E-05	5.00E-05
$f_o$	packing fraction	1	1	0.6
$T_f$	% trans filter	1	1	0.8
$T_i$	% trans imaging	1	1	0.9
$T_o$	% trans optics	1	1	0.9
$\eta_c$	photocathode eff.	0.2	0.2	0.1
$g$	group factor	1	1	1
$N_s$	signal photons	1.7x105	2500	338
$o_o$	electronic noise	55	55	50
$G$	gain	1	1	10,000
SNR	signal/noise	407	34	18

TABLE 1

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TABLE 2

	normal		adenoma	
$\lambda$ (nm)	$\lambda_{ex}=370$	$\lambda_{em}=460$	$\lambda_{ex}=370$	$\lambda_{em}=460$
$\mu_a$	0.9	0.45	2.1	1.1
$\mu_s$	15.0	9.5	8.5	5.9
g	0.9	0.9	0.9	0.9
mucosal thickness (um)	450	450	1000	1000
quantum eff(mucosa)	0.1	0.1	0.8	0.8
quantum eff(submucosa)	0.1	0.1	0.8	0.8

TABLE 3

	Adenoma	Hyperplastic	Blood Vessels
<i>Normalized Intensity</i>			
Normal	100.00±27.5	100.0±18.1	100.00±16.1
Left	54.0±16.3	87.9±16.3	
Center	59.9±16.7	95.9±16.7	75.3±5.1
Right	54.4±17.7	98.1±17.7	
<i>Intensity Ratio (normal/lesion)</i>			
Average	2.0±0.6	1.1±0.2	1.3±0.1
Left	2.0±0.6	1.2±0.1	
Center	1.8±0.5	1.1±0.1	
Right	2.0±0.7	1.1±0.2	

TABLE 4

	Imaging		Single Point	
	Normalized Intensity	Intensity Ratio	Normalized Intensity	Intensity Ratio
<b>Left Adenoma</b>				
Left	65	1.54	58	1.72
Center	74	1.35	74	1.35
Right	71	1.41	65	1.54
Avg	70	1.43	66	1.54
<b>Right Adenoma</b>				
Left	61	1.64	57	1.75
Center	81	1.23	67	1.49
Right	59	1.69	52	1.92
Avg	67	1.52	59	1.72

TABLE 5

	endoscope		probe	
	normal	adenoma	normal	adenoma
d(mm)	20	20	2	2
$\theta_m$ (deg)	4	4	12.7	12.7
$n_0$	1.0	1.0	1.4	1.4
$n_1$	1.4	1.4	1.4	1.4
370	2.8E-02	2.8E-02	4.2E-03	6.2E-04
460	8.8E-05	2.9E-05	2.8E-03	9.7E-04
Ratio	3.0		2.9	

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5           Figure 13 presents a schematic outline of the system  
which has been demonstrated in clinical practice in a  
format which will allow comparison with the improved  
systems to be described below. The embodiment shown uses  
an ultraviolet laser source 200, switched by a shutter 202  
10       and focused with a lens 204 into a fused silica fiber probe  
206 inserted into a biopsy channel of an endoscope 208 to  
deliver it to a tissue site 210 so that it can illuminate  
the tissue over an area 212. The UV illumination thus  
comes from an aperture 214 which is different from the  
15       endoscope's own illumination ports 216. In the dual-  
channel Pentax endoscopes used in the clinic this procedure  
leaves one biopsy channel 218 free.

          The endoscope camera 220 obtains its white light  
illumination through its own fiberoptic illuminator 222  
20       from a broadband Xenon arc lamp 224 and collection optics  
226. A non-standard shutter 228 under computer 230 control  
232 is attached to allow the white light illumination to be  
turned off while fluorescence images are being taken. The  
fluorescence image signal 234 is processed by the  
25       endoscope's video processor 236 to produce a standard video  
signal 238 which is digitized by a framegrabber in computer  
230. The processed image signal 240 with its information  
on the state of the observed tissue is sent to monitor 242.  
The entire diagnostic procedure is initiated by a foot  
30       switch 244 attached to the computer by a cable 246.

          Figure 14A shows an design for the fluorescence  
imaging system which eliminates the tendency of the  
previous system to identify shadows in the image as regions

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5 of dysplasia. The improved design uses a 100W mercury (Hg) arc lamp light source 302, dichroic mirrors 304 and 306, wavelength filters 308 and 310 and rotating shutters 312 and 314 to provide precisely-timed, tissue-illumination pulses in two separate wavelength bands. The first  
10 wavelength band is centered on the near-ultraviolet (365 nm) mercury resonance line and is used to obtain the UV autofluorescence image. The second wavelength band is at the red end of the visible spectrum and is used to obtain a simultaneous or near-simultaneous, reflectance image for  
15 the purpose of identifying shadows and the extent of the UV illumination field.

A reflectance (non-fluorescing) image taken with an endoscope camera system measures the brightness of the tissue surface 316 in its field of view. To the extent that  
20 the tissue surface is a Lambertian (non-specular) reflector (generally the case) this image indicates the distance of the tissue from a single illumination source (or a weighted distance from multiple sources). If these illumination sources are not in the direct line-of-sight from the camera  
25 to the tissue source there will be shadows. A reflectance image can thus be used to measure both the UV illumination 318 at the tissue surface and the presence of shadows in the fluorescence image as long as the UV illumination and the visible illumination emanate from the same aperture 320  
30 with the same angular divergence. Note that this condition can be satisfied either by a two-color illumination fiber 322 passed through a biopsy channel of an endoscope 324 or by the two-color illumination being passed through the

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5 illumination bundle 326 of the endoscope. A shutter 328 switches off the normal white-light illumination of the endoscope while the two diagnostic images are being obtained. The closing of shutter 328 under computer control 329 occurs at the same time as the opening of  
10 shutter 330 by control line 331. This action enables the two-color light to reach the fiber 322 and thus the tissue 316.

The algorithm for using the visible reference image along with the fluorescence image is as follows. The video  
15 signals 332 from the CCD camera at the distal tip of the endoscope 324 are converted by the video processor 334 to a standard NTSC color video signal 336 and sent to a video framegrabber in computer 338. The two images are first corrected for the gamma factor applied to the video signal  
20 by the video processor, to insure that the digitized images acquired by the framegrabber in the computer are linear measurements of the tissue surface brightness. This is accomplished in real time by the framegrabber input look-up table. The two images are then normalized to their peaks,  
25 which will generally be a region of non-dysplastic tissue in the visual field. This normalizes the two illumination fields. On a pixel-by-pixel basis the fluorescence image value is then divided by the visible reference image value. If the ratio falls below a predetermined threshold  
30 (typically one-half to one-third) then that pixel in the image represents a region of reduced fluorescence which is indicative of dysplasia. This pixel can then be set to a false color state in an output video signal 340 sent to a

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5 monitor 342 to indicate to the clinician the probability  
of dysplasia. A prior threshold requirement on both images  
insures that the ratio obtained is significant and  
eliminates false color output in regions of shadow or low  
illumination at the edges of the video field. This entire  
10 operation occurs for every depression of the footswitch 344  
which is connected to the computer through cable 346.

In the improved design both the UV-excitation light  
pulse and the visible-reference light pulse are delivered  
to the tissue through the same optical fiber 322 inserted  
15 through a biopsy channel of the endoscope. The condition  
that the two illumination sources have the same angular  
distribution is assured by the design of the light  
collection apparatus shown in Figure 14A. A single Hg arc  
lamp 302 is used as the source of both wavelengths. A  
20 dichroic mirror 304 reflects the UV portion of the spectrum  
and transmits the visible portion. Filters in each path  
further refine the bandwidth of the two beams. The UV  
filter 308 must reject visible light to a high degree since  
the efficiency of the 460 nm tissue fluorescence is only  
25 about 0.1%. The filter 310 in the red path is less  
critical but the chosen center wavelength should avoid  
hemoglobin absorption bands to provide the best reference  
image. Note that the design of the beam splitting optics  
and beam combining optics have an even number of  
30 reflections in both the UV and visible arms. This assures  
that any angular deviations of the output beams due to  
motion of the lamp track each other. It also makes the  
directions of the output beams invariant under translations



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5 and small rotations of the beam splitting and recombining optics as a whole.

In the improved source design the current through the Hg lamp 302 is boosted at appropriate moments to increase the lamp output power for the UV exposure. This allows a  
10 larger area of tissue to be scanned for dysplasia in a single image. The data in Figure 14B show that the UV output power from a 100 W Hg lamp is a linear function of its input power to at least a factor of 3 over its nominal rated power. Since the lamp discharge maintains a constant  
15 voltage drop across the arc regardless of current, the lamp output power is essentially proportional to current. At least 50% power to the lamp must always be maintained, however, to keep the mercury in the vapor phase. The lamp power supply 348 in the improved fluorescence system  
20 utilizes a DC current section to maintain the idle current and a computer-controlled 350, pulsed current section which can rapidly switch in multiple constant-current sources to vary the output power of the lamp as required by the imaging system. If the idling power is kept below the rated  
25 power and the current pulses are kept to a sufficiently small duty factor, then the pulsed UV output can be sustained continuously.

The rotating shutter in the UV path 312 and in the red path 314 of the light source are designed to provide pulsed  
30 illumination light according to the timing diagram in Figure 14C. This diagram shows how this fluorescence imaging system is used with a monochrome camera, which uses a xenon arc lamp 352 for illumination and a rotating blue-

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5 green-red filter wheel 354 to synthesize a color image from  
3 monochrome images. In this type of system a pulse of blue  
light first illuminates the tissue for about 6 milliseconds  
and the resulting tissue reflectance image is digitized for  
the next 6 milliseconds. The illumination must be turned  
10 off during the readout period because the monochrome camera  
used continues to collect photo current in the pixels as  
they are line shifted to the readout electronics.  
Illumination during the readout causes a smearing artifact  
in the image. The UV illumination is switched in during the  
15 normal blue exposure period and the red light illumination  
is switched in during the red exposure period. The green  
exposure period is not generally used but could be used to  
obtain an additional reference image or an additional UV  
fluorescence image. The shutters are timed to the video  
20 acquisition system using an LM1881 Video Sync Separator  
circuit to develop an even/odd frame synchronization pulse  
356 from the standard composite video output signal. Phase-  
locked-loops (PLL) 358 and 360 synchronize the phase of the  
chopper wheels to this signal by varying a voltage to their  
25 DC driving motors 362 and 364. This signal is also used to  
synchronize the current pulser 348. In the schematic of  
Figure 14A the chopper wheels are shown in collimated  
portions of the beam. In practice, these chopper wheels are  
placed at an internal focal point in the two arms of the  
30 optics train (not illustrated) to provide for fast rise and  
fall times for the light pulse.

Note that the dual-wavelength illumination method can  
also be used with standard, color-CCD camera endoscopes. In

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5        this case the UV light illumination and red light reference  
illumination are present simultaneously. The UV-induced  
fluorescence (primarily at 460 nm) is then detected by the  
blue-responsive pixels in the CCD camera and the reference  
reflectance image is detected by the red-responsive pixels.  
10      Note that the visible blue light must still be removed from  
the diagnostic illumination so as not to decrease the  
contrast of the fluorescence image. The slight amount of  
red tissue fluorescence seen in dysplastic tissue due to  
the UV excitation is much smaller than the level of direct  
15      red illumination. The slight increase also acts to reduce  
the fluorescence/reference ratio which properly increases  
(slightly) the measured probability of dysplasia.

Figure 15 shows a preferred embodiment of the  
fluorescence imaging system in which dual wavelength  
20      illumination capability as well as white-light illumination  
capability is built into the video endoscope system itself  
400. This requires the illumination bundle 402 of the  
endoscope 404 to be transmissive at UV wavelengths which is  
not usually the case with current commercial systems. Such  
25      a design fulfills the design requirement that the UV-  
excitation and visible-reference illumination emanate from  
the same aperture or apertures 406. Such a system would  
also be easier for an operator to use since the  
illumination fiber would not have to be threaded down  
30      through a biopsy channel 408 and those channels would be  
free for their standard uses. The tissue surface 410 would  
be illuminated over a larger area 412 with fewer shadows  
since dual illumination ports 406 are standard. The video

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5        signal 414 would be processed by the endoscope system 416,  
formatted into a standard signal 416 and processed by the  
computer 420. The output video signal with the false color  
overlay 422 would be sent to a monitor 424 for the  
clinician to see in real time. The diagnostic illumination  
10        would be initiated by a footswitch 426 connected by a cable  
428 to the computer 420 with the light pulses controlled by  
signal lines 430 and 432 to the shutters in the light  
source.

15        While this invention has been particularly shown and  
described with references to preferred embodiments thereof,  
it will be understood by those skilled in the art that  
various changes in form and details may be made therein  
without departing from the spirit and scope of the  
invention as defined by the appended claims.

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5

Claims

1. A method of fluorescence imaging comprising:  
    providing an endoscope having an optical guide  
    that is optically coupled a first light source and a  
    second light source, the endoscope having an image  
10     sensor at a distal end;  
    collecting a reflectance image with the image  
    sensor and generating a reference;  
    collecting a fluorescence image with the image  
    sensor; and  
15     processing the fluorescence image with the  
    reference to provide a processed fluorescence image.
2. The method of Claim 1 further comprising providing an  
    image sensor having a filter to remove ultraviolet  
20     light from light returning from tissue.
3. The method of Claim 1 wherein the image sensor has a  
    sensitivity in the ultraviolet region that is less  
    than half of the sensitivity of the sensor in a  
25     visible region.
4. The method of Claim 1 wherein the reference corrects  
    intensity for shadows in the fluorescence image.
- 30     5. The method of Claim 1 wherein the fluorescence image  
    is non-intensified.
6. The method of Claim 1 wherein the first light source

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5 is a broadband source.

7. The method of Claim 1 wherein the second light source generates a wavelength in the range of 350nm to 420 nm.

10 8. The method of Claim 1 further comprising compensating for shadows on a tissue surface to be imaged.

15 9. The method of Claim 1 wherein the first and second light source are optically coupled to the same optical guide.

10. The method of Claim 1 further comprising providing a shutter coupling each light source to an optical fiber.

20 11. The method of Claim 1 further comprising imaging dysplasia on a tissue surface.

12. A fluorescence imaging system comprising:  
25 an endoscope;  
a light source coupling to an optical guide extending through the endoscope; and  
an imaging sensor at a distal end of the endoscope that detects a fluorescence image and  
30 reflectance image of tissue.

13. The imaging system of Claim 12 wherein the light source comprises a first broadband light source and a

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5           second narrow band light source.

14.   The imaging system of Claim 13 wherein the narrow band light source emits light having a wavelength in the range of 350nm to 420nm.

10

15.   The imaging system of Claim 12 further comprising a fiber optic device optically coupled to the light source.

15

16.   The imaging system of Claim 12 further comprising a processor that processes the reflectance image and the fluorescence image and generates compensated fluorescence image.

20

17.   The imaging system of Claim 12 further comprising a shutter positioned along an optical path between the light source and an optical fiber extending through the endoscope.

25

18.   The imaging system of Claim 12 wherein the image sensor has reduced sensitivity in an ultraviolet spectral region relative to sensitivity in a visible spectral region.

30

19.   The imaging system of Claim 12 wherein the image sensor comprising a filter reducing image sensor sensitivity below 400nm.

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- 5        20. The imaging system of Claim 18 wherein the sensor sensitivity in the ultraviolet spectral region is less than one half of the sensitivity in the visible region.



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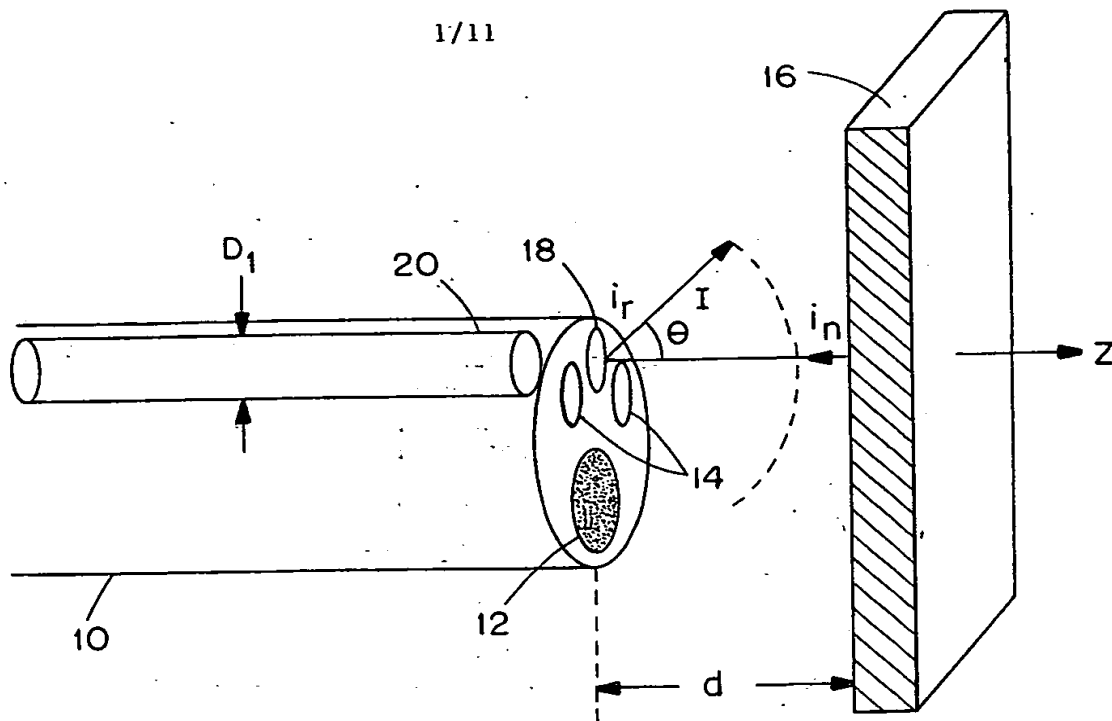


FIG. 1

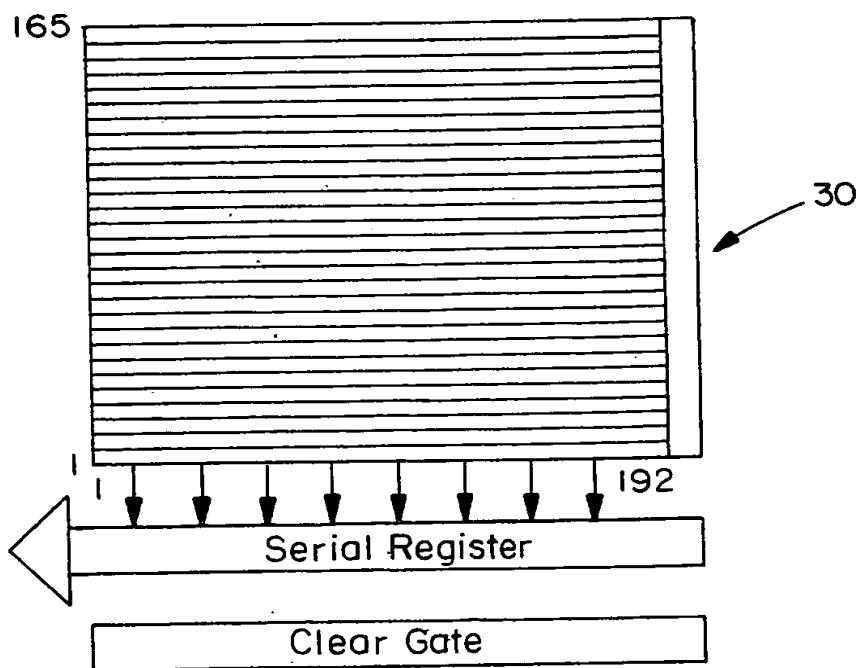


FIG. 2

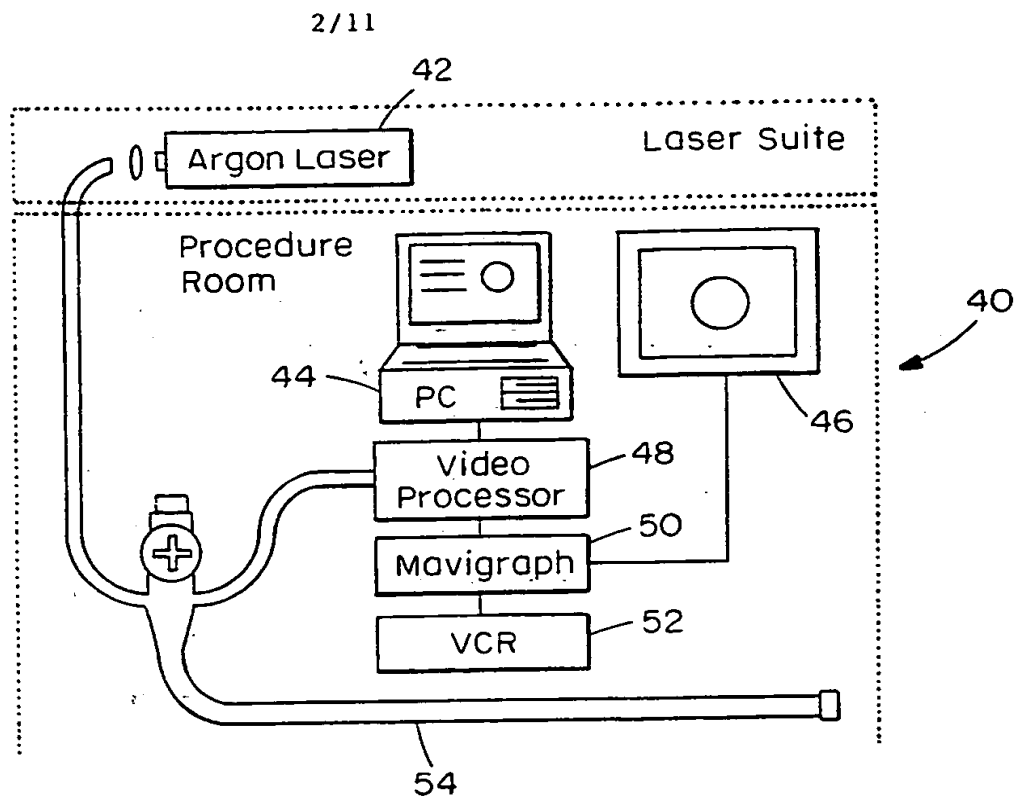


FIG. 3

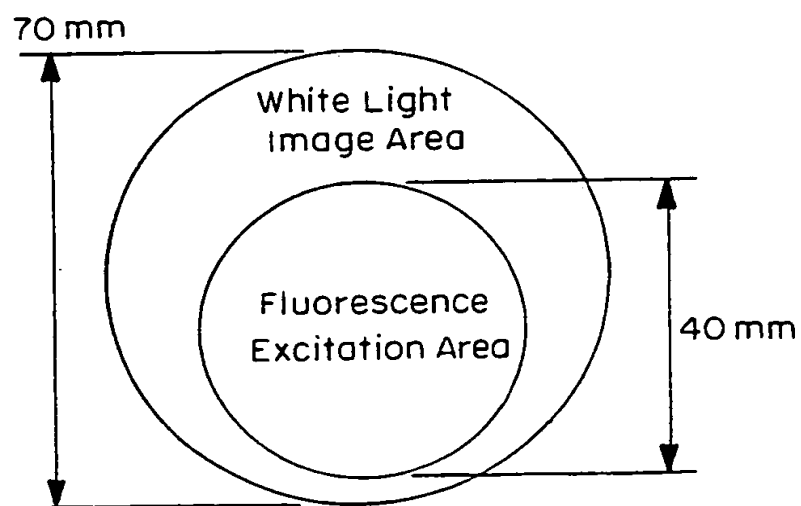


FIG. 4

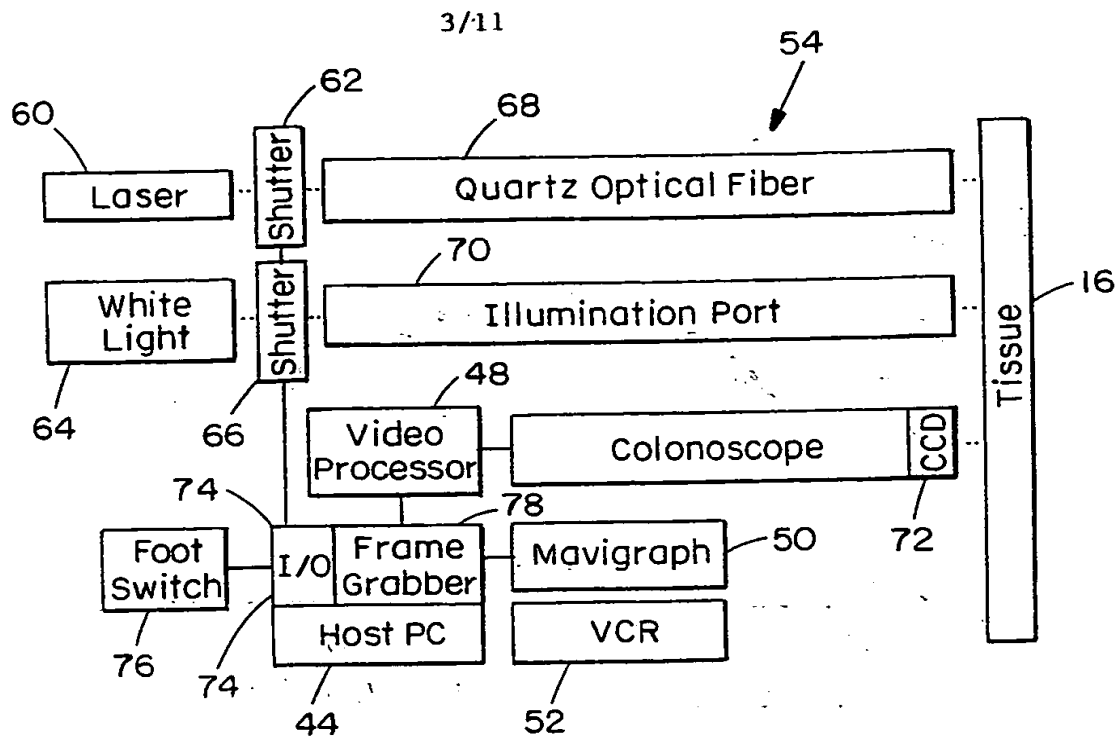


FIG. 5

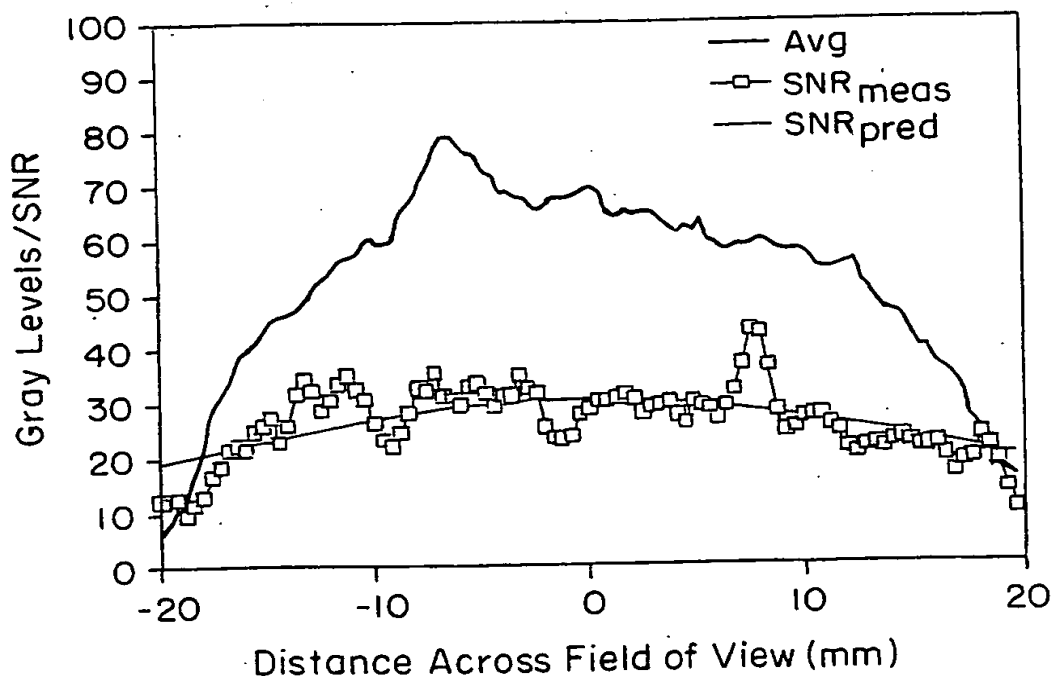
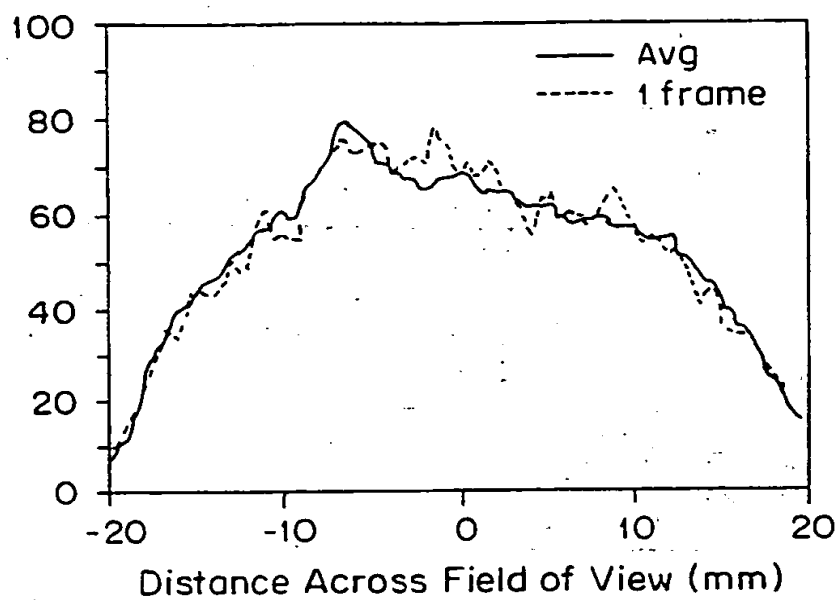
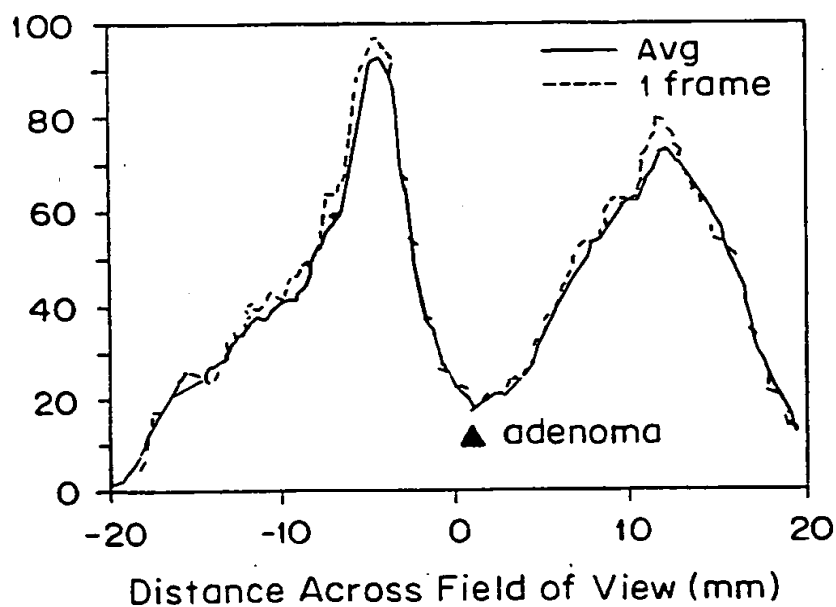


FIG. 6

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**FIG. 7A****FIG. 7B**

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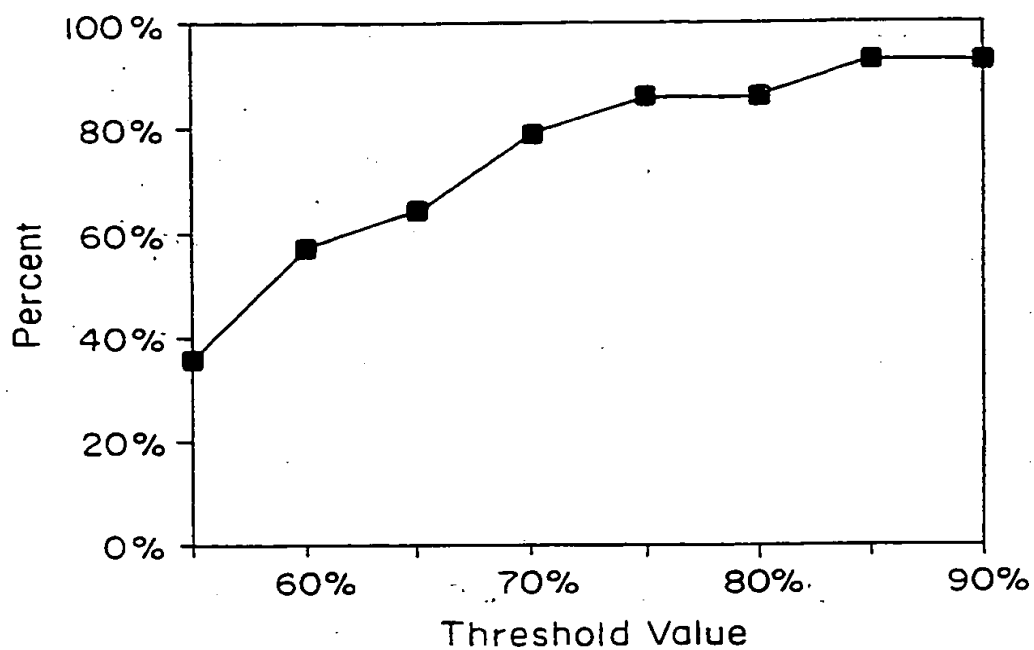


FIG. 8

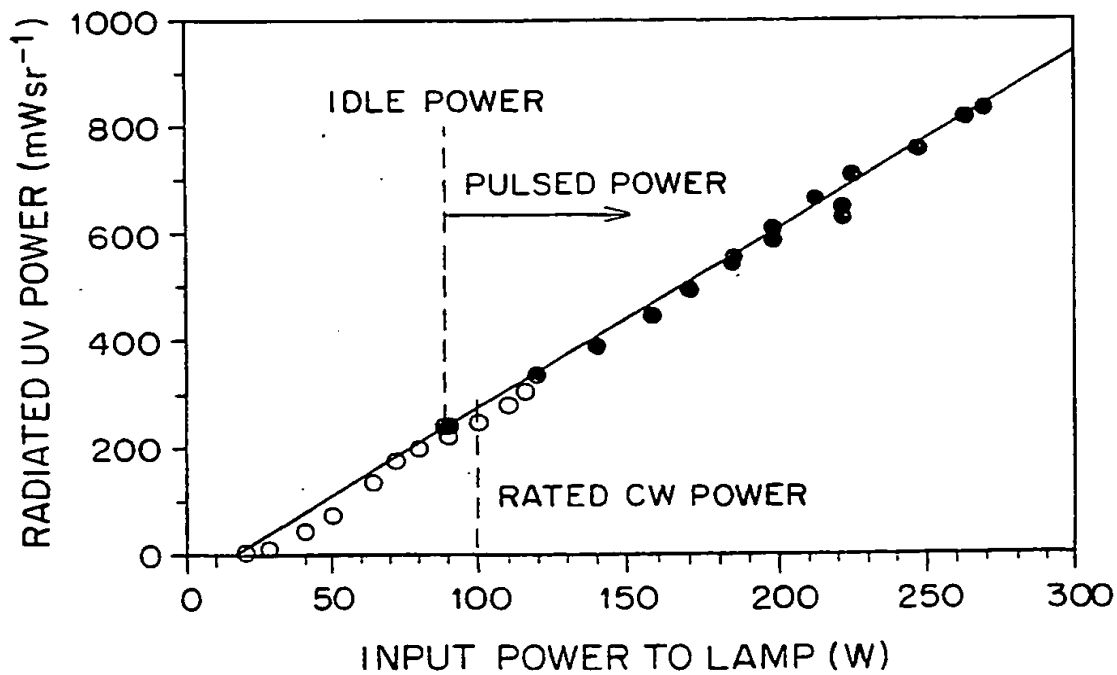


FIG. 14B

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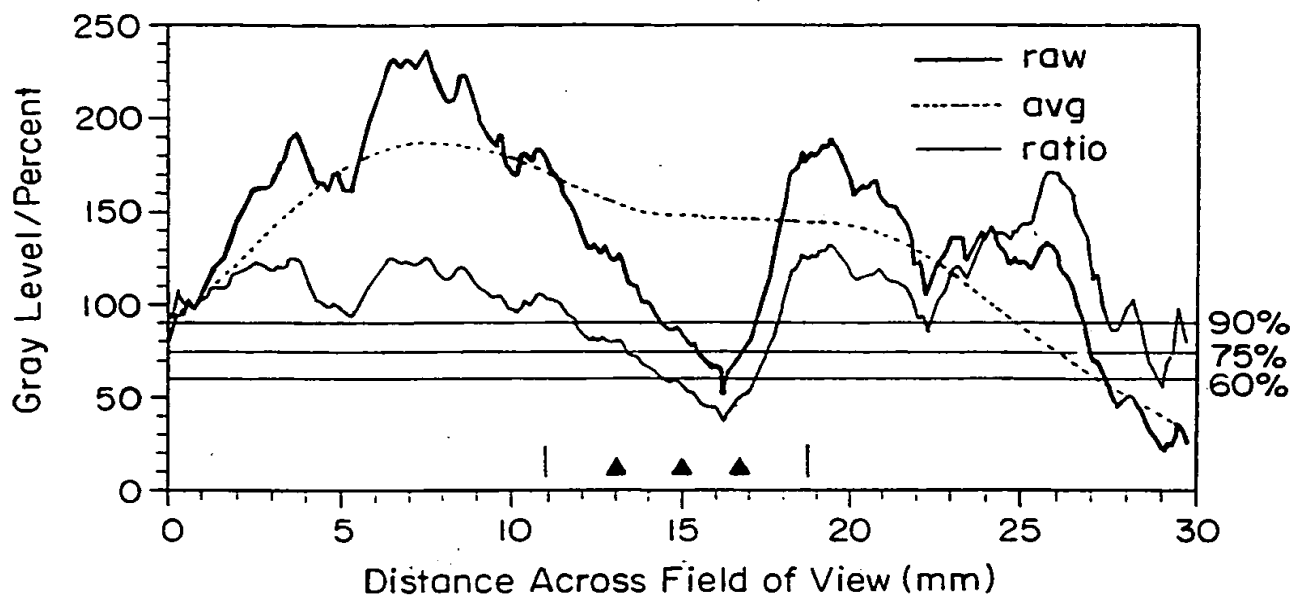


FIG. 9

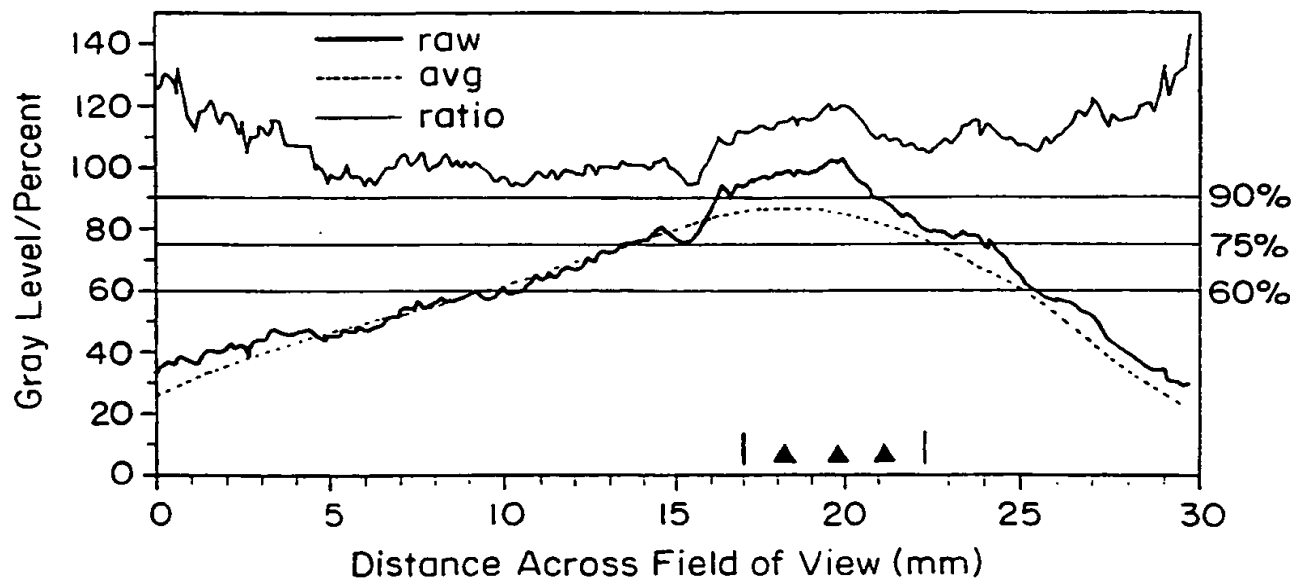


FIG. 10

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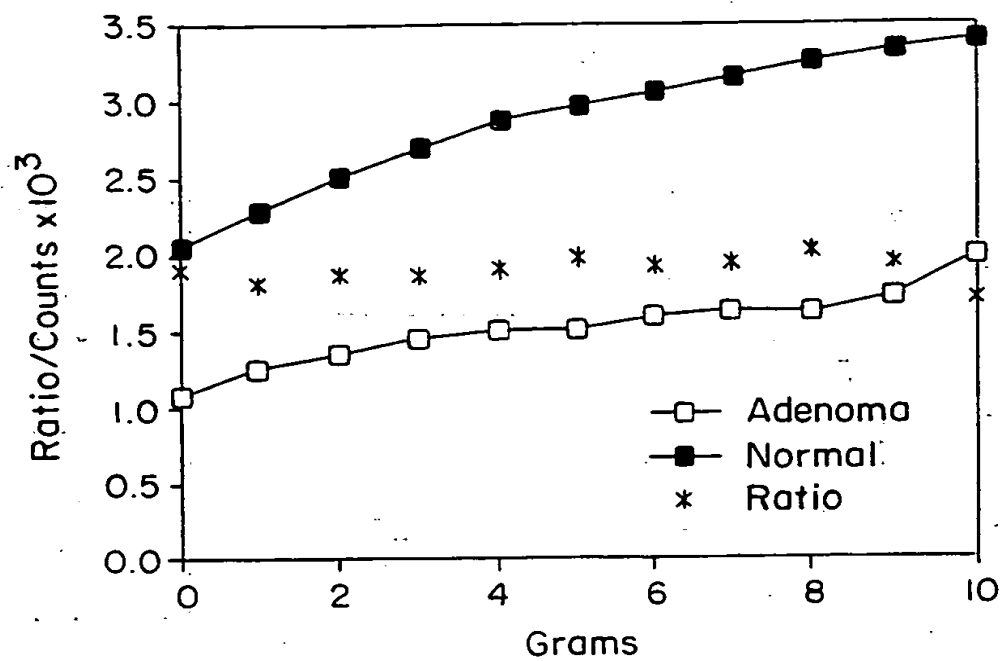


FIG. 11

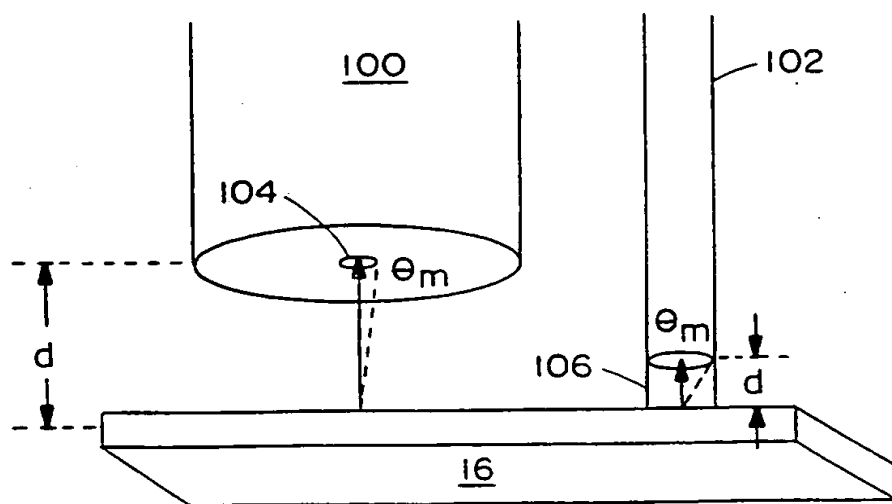


FIG. 12

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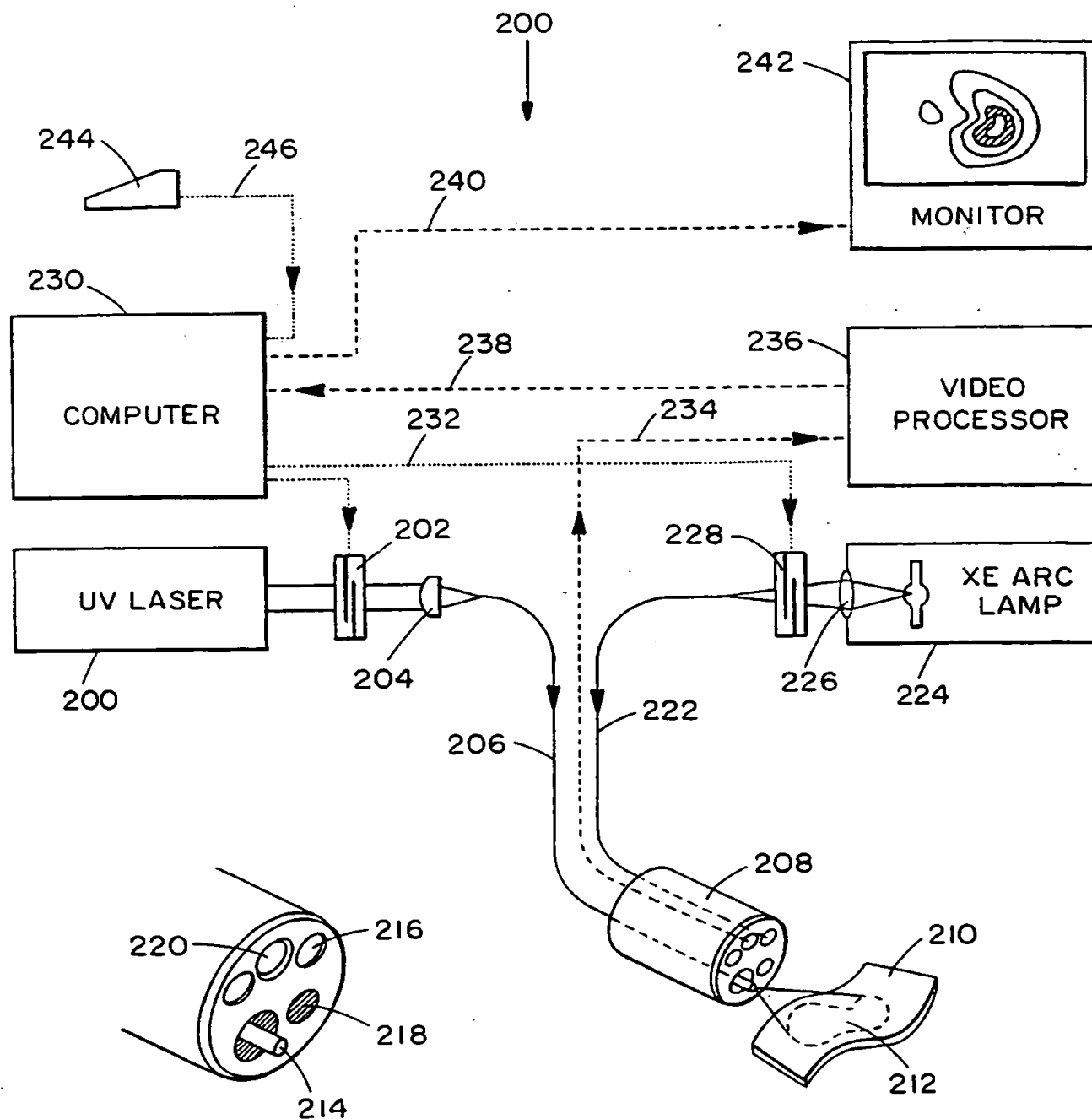


FIG. 13



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300

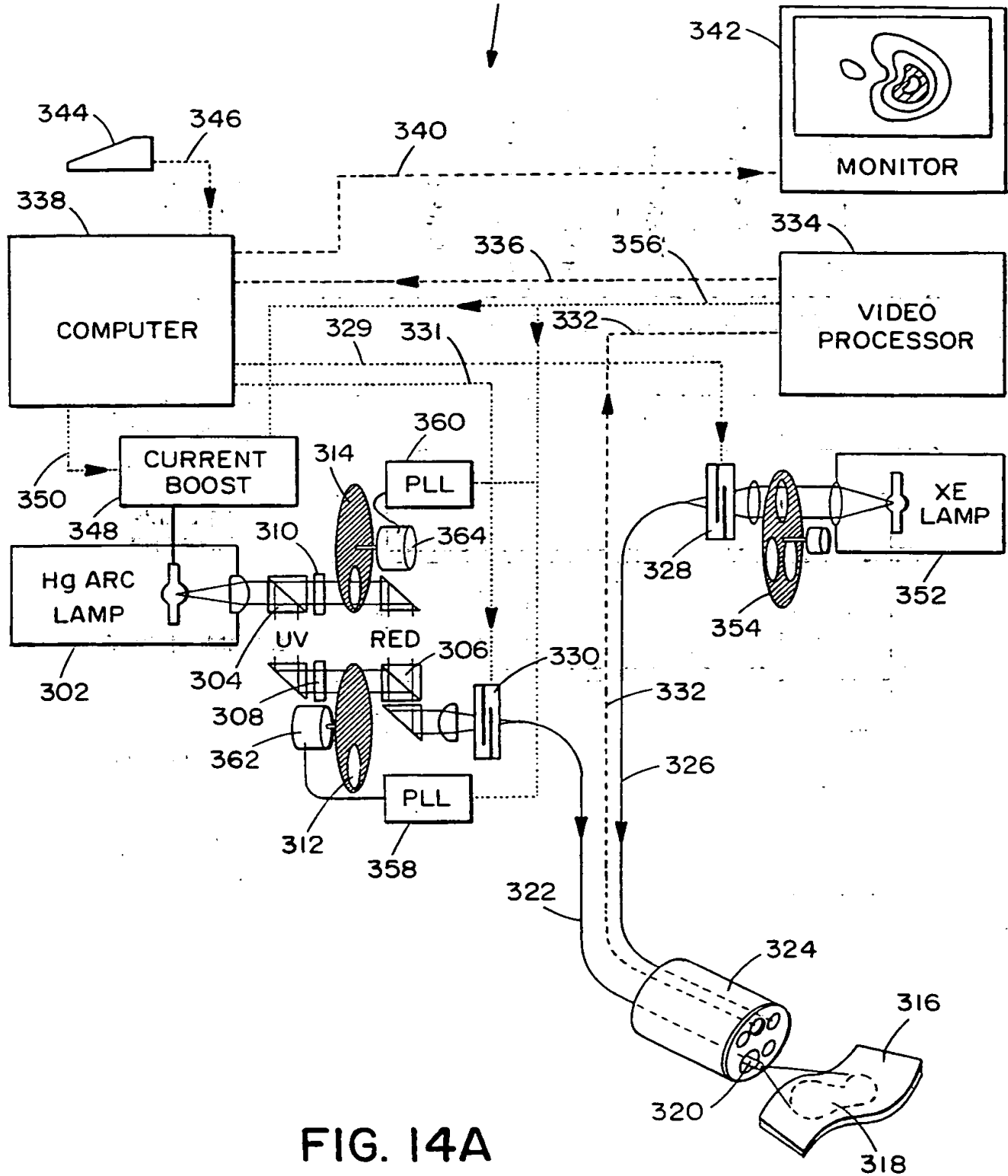


FIG. 14A

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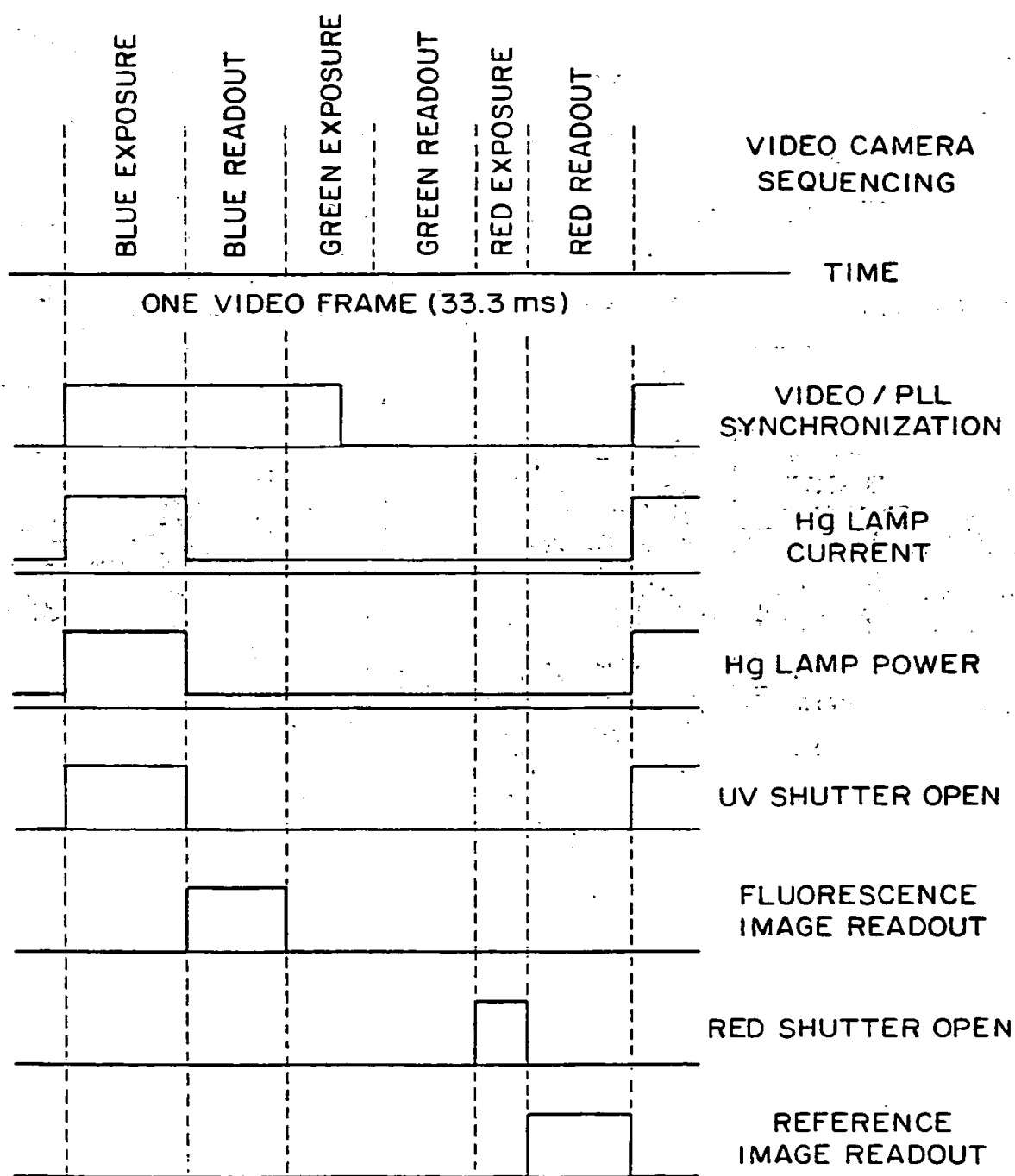
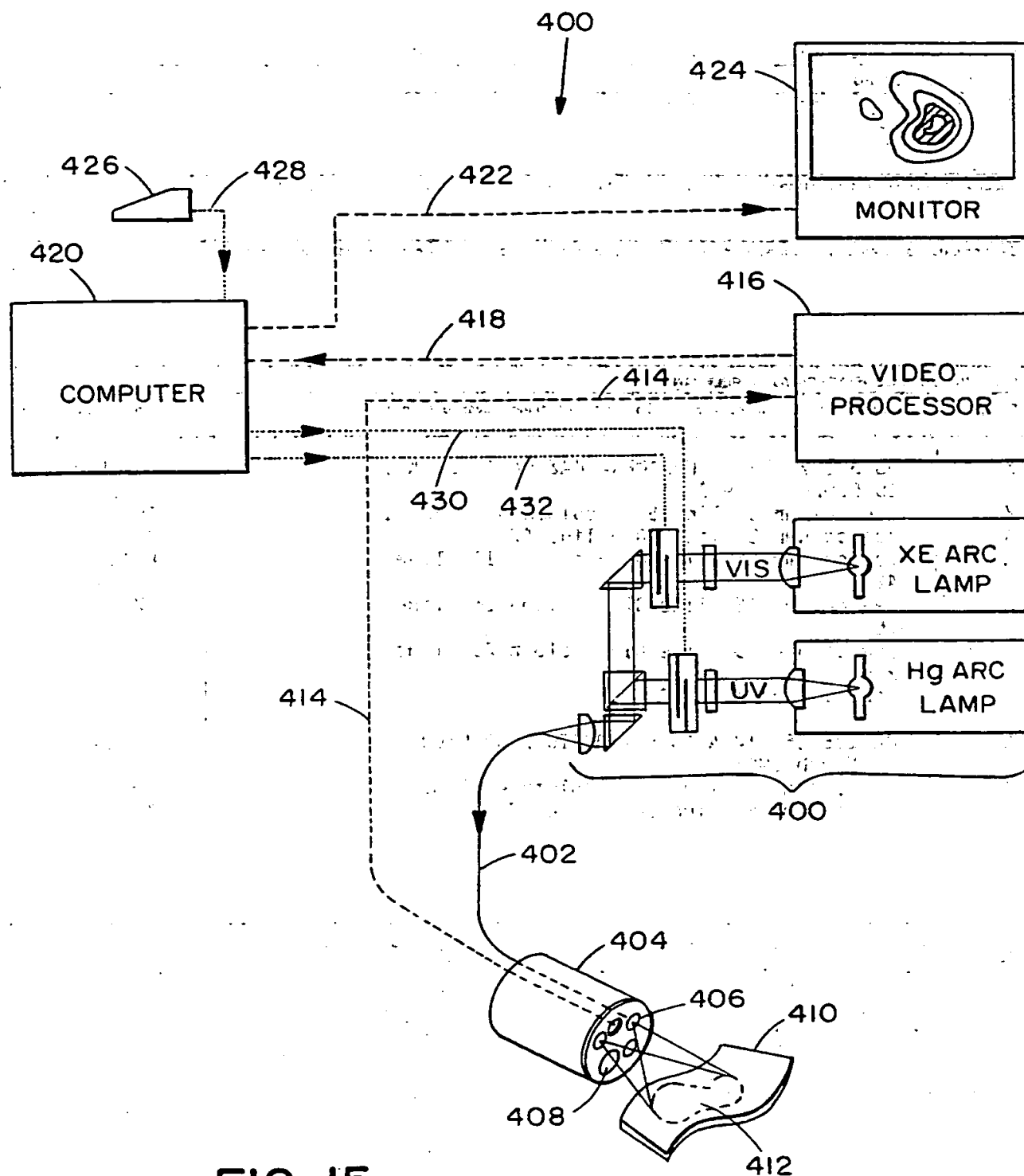


FIG. 14C

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/01723

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 187 572 A (NAKAMURA KAZUNARI ET AL) 16 February 1993	1,2,5,6, 9,12,13
X	see column 3, line 67 - column 4, line 31	16
A	see column 5, line 30 - line 47	3,4,7
A	see column 11, line 43 - column 12, line 42	8,10,11, 14,15
A	see column 15, line 37 - column 16, line 16	17-20
	see column 19, line 61 - column 20, line 20	
	see figures 1,11,28	
X	DE 195 35 114 A (ASAHI OPTICAL CO LTD) 28 March 1996	12,15, 16,19
A	see column 3, line 38 - column 4, line 3	1,2,5-7
A	see column 5, line 14 - line 57	11,13
	-/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Δ" document member of the same patent family

Date of the actual completion of the international search

17 May 1999

Date of mailing of the international search report

25/05/1999

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Martelli, L

# INTERNATIONAL SEARCH REPORT

In International Application No

PCT/US 99/01723

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 19148 A (ADAIR EDWIN LLOYD) 12 November 1992	12,15
A	see page 3, line 18 - page 5, line 3	1,2,5-7
A	see page 8, line 32 - page 11, line 17	11,13, 14,16
A	----- US 5 452 723 A (WU JUN ET AL) 26 September 1995 cited in the application see column 3, line 23 - line 32 see column 4, line 45 - column 5, line 14 see column 6, line 8 - line 15 see figure 1 -----	1,5,6, 9-13, 15-17

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/01723

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5187572 A	16-02-1993	JP 5084218 A DE 4136034 A	06-04-1993 07-05-1993
DE 19535114 A	28-03-1996	JP 8140928 A JP 9070384 A JP 9066023 A JP 8140929 A	04-06-1996 18-03-1997 11-03-1997 04-06-1996
WO 9219148 A	12-11-1992	US 5143054 A CA 2065976 A DE 69217216 D DE 69217216 T EP 0585321 A JP 6506855 T US 5251613 A	01-09-1992 07-11-1992 13-03-1997 31-07-1997 09-03-1994 04-08-1994 12-10-1993
US 5452723 A	26-09-1995	NONE	